

AMERICAN CHEMICAL JOURNAL

Contributions from the New York Agricultural Experiment Station.

I. — A STUDY OF SOME OF THE SALTS FORMED BY CASEIN AND PARACASEIN WITH ACIDS: THEIR RELATIONS TO AMERICAN CHEDDAR CHEESE.

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The chemical changes taking place during the early stages of the manufacture of cheese have hitherto been little investigated or understood. This is particularly true of the kind of cheese most extensively made in the United States and Canada, commonly called American cheddar cheese. One of the first recognized changes occurring in the process of cheese-making is that produced by the action of rennet upon milk-casein, resulting in the formation of an insoluble curd. The action of rennet, according to Hammarsten's¹ explanation, splits milk-casein into two different compounds, one, the insoluble curd, called paracasein, formed in large proportions; the other, soluble, albumose-like, called whey proteid, found in small proportions. While Hammarsten's view is still held by many, there is growing evidence² that the albumose-like

¹ Maly: *Jahrb. d. Thierchemie*, **2**, 118 (1872); **4**, 135 (1874).

² Fuld: *Beiträge zur chem. Physiol.*, **2**, 169 (1902).

proteid found in whey is not a result of the action of rennet, but that it can be produced by actual proteolytic digestion of casein, due to enzymes either present in the milk itself or introduced with the rennet (as pepsin or Glaessner's pseudo-pepsin). Whatever we may ultimately find the exact nature of the action of rennet on milk-casein to be, it is not the only factor at work during and after the formation of the insoluble curd, for there is present at least one other active process during the early stage of cheese-making that has much to do with the production of a "good curd," judged from the practical standpoint of a cheese-maker.

In cheddar cheese-making, one of the first operations is to "ripen" the milk, previous to adding rennet. This is accomplished by allowing the milk to stand for some time at a temperature of about 30° C., or the operation may be hastened by adding to the milk special cultures of acid-producing organisms. This process, commonly known as the "ripening of milk," has for some time been recognized as an acid-producing form of fermentation; that is, a fermentation caused by the action of lactic-acid organisms on the milk-sugar, resulting in the formation of lactic acid. In ripening milk, the cheese-maker increases the quantity of acid in the milk, not only before adding acid, but continuously throughout the rest of the process of manufacture.

The amount of acid in cheese-curd is roughly measured in a mechanical way by means of the so-called "hot-iron test." When a piece of curd is pressed against a hot iron and then drawn away carefully, fine, silky threads are formed, adhering to the iron. This phenomenon is closely associated with the formation of acid in the cheese-vat, and the length of the strings shown by the hot-iron test is utilized as a crude measure of the amount of acid present and as an indication of when to perform certain operations. Thus, when the curd strings on a hot iron to the length of one-eighth of an inch, the whey is removed from the curd, after which the curd is "packed" in a pile and allowed to lie until it has passed through the so-called "matting" or "breaking-down" pro-

cess, when it furnishes strings an inch or more in length by the hot-iron test. When this stage is reached, the remaining steps of the manufacturing process are at once completed, such as milling, salting, and putting the curd in molds for pressing.

While it has generally been supposed that the presence of some acid, presumably lactic acid, in the cheese-making process is, in some way or other, responsible for the most important changes taking place, such as the shrinking of the curd, the acquired ability to form strings on a hot iron, and the changes in the appearance and plasticity of the curd, no one has ever been able to show in what way these changes were brought about by acids. It has been commonly supposed that the observed changes were purely physical in character and were not the result of chemical changes in the curd. In the following pages it is our purpose to consider more fully than has been previously done the real function of acids in relation to the important changes taking place in cheese-curd during the cheddar process of cheese-making.

Since Sjöquist's¹ investigation, we have learned that proteids can form salts with acids and bases, and this subject has been considerably developed in the last few years. Bugarszky and Liebermann² have shown that egg albumin forms with hydrochloric acid a salt analogous to ammonium chloride, which they call albuminum chloride. Osborn³ has also shown that edestin, the globulin of hempseed, is capable of combining with acids in several different proportions, forming salts having different properties. Panormoff⁴ also has recently described definite compounds formed by egg albumin with different acids. So far as we are able to learn, no similar work with milk-casein or with paracasein (milk-casein coagulated with rennet and constituting a large proportion of cheese-curd) has been done. The nearest any one has come to touching this specific line of work was in the case of Danilewsky,⁵ who,

¹ Skandin: Arch. für Physiol., **5**, 277 (1894).

² Pflüger's Archiv., **72**, 51 (1898).

³ Ann. Rep. of Conn. Agr. Expt. Sta., **23**, 402 (1900).

⁴ Jour. d. Russ. Phys. Chem. Ges., **31**, 556.

⁵ Ztschr. physiol. Chem., **7**, 433 (1883).

in the preparation of what he called pure casein, by the use of hydrochloric acid, actually obtained a mixture of the salts of this proteid, one of which is soluble in boiling 50 per cent alcohol, while the other is insoluble. From this behavior he concluded that milk-casein consists of two proteids. Reference will be again made to this.

It may be stated in this connection that in 1882 Weidmann¹ dissolved from Emmenthaler cheese, by hot alcohol, a substance which he called caseoglutin. This substance has been mentioned in the literature a few times since and has been regarded as a peptone-like body, but no one has ever investigated it and nothing has been known of its real nature. We believe that we have found a satisfactory explanation showing its character, which we will give later in this paper.

In our early work relating to the study of the chemical changes taking place in cheese during the process of ripening or curing, we mixed the cheese thoroughly with sand by grinding in a mortar, and completely extracted this with water at 55° C. The residue, insoluble in water, was then treated with a 10 per cent solution of sodium chloride at 40° C. for the purpose of removing any heterocaseose formed during the ripening process. Chittenden² had shown that, in a peptic digestion of casein, heterocaseose was formed only in small amounts. When, by our extraction of fresh or partly ripened cheese with dilute salt solution, we obtained amounts of proteid representing often as high as 40 per cent of the total nitrogen present in the cheese, it became apparent that we were dealing with some compound other than heterocaseose.

EXPERIMENTAL PART.

Occurrence of the Salt-soluble Product in Cheese Made in the Presence of Chloroform with and without Acid.

(1) *Without Acid.*—Cheeses were made from milk which had been previously heated to 98° C. for the purpose of de-

¹ Landwirtsch. Jahrb., 1882, p. 587.

² Studies in Physiol. Chem., Yale Univ., 2, 156 (1885-6).

stroying all enzymes and bacterial forms present in the milk. After cooling the milk to the temperature employed in cheese-making, chloroform was added to the milk to the extent of 4 per cent by volume, in order to prevent any bacterial activity. As the ability of milk-casein to coagulate with rennet is impaired by heat, this property was restored, in one case, by the addition of a small amount of calcium chloride, and, in the other case, by passing a stream of carbon dioxide through the milk for half an hour. The cheeses, made from the milk thus treated, were left in press over night and were then examined at once for the salt-soluble product. The determination was made as follows: Twenty-five grams of the fresh cheese were ground with sand and extracted with several portions of distilled water at 55° C., until the extract amounted to 500 cc. The residue was then similarly extracted with a 5 per cent solution of sodium chloride. The amount of nitrogen in 50 cc. of this salt extract was determined by the Kjeldahl process. Table I. gives the results obtained. The figures in parentheses indicate the serial numbers of the different cheeses.

(2) *With Acid.*—Other cheeses were made exactly like the preceding, except that lactic acid was added to the milk to the extent of 0.2 per cent, by weight, of the milk. An examination of these cheeses, made at once after they were taken from the press, gave the results stated in Table I.

Table I.—Amount of Nitrogen in Salt-soluble Extract, Obtained from Cheeses Made with and without Lactic Acid.

	Nitrogen, expressed as percentage of total nitrogen in cheese.		Nitrogen, expressed as percentage of cheese.	
	Made with addition of calcium chloride.	Made with addition of carbon dioxide.	Made with addition of calcium chloride.	Made with addition of carbon dioxide.
Without acid	2.44 (44)	5.24 (49)	0.76 (44)	0.132 (49)
	2.90 (47)	5.72 (50)	0.76 (47)	0.148 (50)
With acid	27.88 (45)	29.80 (48)	0.87 (45)	0.700 (48)
	26.62 (46)	22.90 (51)	0.84 (46)	0.754 (51)

An examination of this table shows that the amount of nitrogen, in the form of the salt-soluble product, was increased

very greatly by the action of the added lactic acid. The cheeses made without acid show small amounts of this salt-soluble product. In the case of Nos. 44 and 47, the salt-soluble product found may possibly be attributed to the slight amount of lactic acid formed in the milk before it was heated, or it may be that neutral paracasein itself is slightly soluble in a dilute solution of salt. There is some basis for this latter supposition, for, in the case of milk taken directly from a cow and immediately etherized and made into curd, these conditions excluding all possible formation of acid, there still appears to be a slight solubility of the neutral paracasein in dilute salt solution.

In the case of Nos. 49 and 50, somewhat more salt-soluble product is found than in 44 and 47. This is undoubtedly due to the effect of the carbon dioxide used, even so weak an acid probably having some limited tendency to form with paracasein a salt-soluble product.

Occurrence of the Salt-soluble Product in Normal Cheese.

In the many normal cheddar cheeses thus far examined by us, this salt-soluble substance is always found. The amounts vary, but the factors causing the variations have not been fully studied yet. For example, the amount of milk-sugar retained in the cheese is one cause of such variations: likewise, any condition affecting the quantitative conversion of milk-sugar into lactic acid. Then again, we have not yet obtained satisfactory control of all the details of the quantitative estimation of this salt-soluble product. Its amount varies also with the age of a cheese. In cheese fresh from press, that is about twenty-four hours old, we have found that from 40 to 78 per cent of the total nitrogen is in the form of the salt-soluble substance, and the amount diminishes more or less as the cheese advances in age, as illustrated by the figures in Table II.

Table II.—Amount of Salt-soluble Product in Cheeses at Different Ages.

Serial number of cheese.	Age of cheese.	Salt-soluble nitrogen, expressed as percentage of total nitrogen in cheese.	Salt-soluble nitrogen, expressed as percentage of cheese.	Water-soluble nitrogen, expressed as percentage of total nitrogen in cheese.	Water-soluble nitrogen, expressed as percentage of cheese.
54	1 day	58.7	2.08	6.78	0.240
"	1 mo.	42.4	1.51	19.30	0.718
"	3 mos.	33.4	1.31	26.02	1.020
31-A	2 weeks	40.7	1.50	15.50	0.569
" B	"	42.8	1.58	16.50	0.608
" C	"	37.4	1.38	16.10	0.584
" D	"	40.8	1.49	15.80	0.589
38-A	3 mos.	16.48	0.58	37.22	1.310
" B	"	17.60	0.63	35.48	1.270
" C	"	19.19	0.71	32.16	1.190
" D	"	21.96	0.83	29.90	1.130
38-A	9 mos.	13.43	0.54	53.24	2.140
" B	"	14.29	0.57	47.75	1.910
" C	"	18.46	0.74	45.64	1.830
" D	"	18.44	0.78	40.91	1.730

An examination of Table II. indicates a general tendency for the amount of nitrogen present in cheese, in the form of the salt-soluble product, to decrease as the cheese advances in age.

An examination of the last two columns of figures in the table shows that, as cheese increases in age, the amount of nitrogen, in the form of water-soluble products, increases. The fact that the water-soluble nitrogen in cheese increases at the same time the salt-soluble nitrogen diminishes, strongly suggests that this progressive proteolysis takes place at the expense of the salt-soluble product. However, we are not inclined to assert positively that such a relation exists, until we have perfected to our satisfaction the method for estimating the salt-soluble product. In cheese No. 54, one of our latest experimental cheeses, our method of extraction was under better control, and the figures obtained are entirely consistent

with the view that proteolysis in cheese commences with the salt-soluble product. However, the main point to which we wish to call attention in connection with Table II. is that in freshly-made cheddar cheese the salt-soluble product forms a large part of the nitrogen compounds of the cheese.

Nature of the Salt-soluble Product.

(1) *A Salt of Paracasein.*—In the case of cheeses made with and without lactic acid, in the presence of chloroform, we have seen that there were large amounts of the salt-soluble product formed when lactic acid was used, and only small amounts when no acid was used. We have also seen that, in the case of normal cheddar cheese, the salt-soluble product occurs in large proportions, increasing in quantity as the milk-sugar disappears, or, in other words, as the amount of lactic acid increases. It, therefore, appeared to us that, in this salt-soluble product, we were dealing with some compound of paracasein and lactic acid, probably a paracasein lactate.

(2) *Artificial Preparation of Paracasein Lactate.*—It seemed desirable to attempt the artificial preparation of the salt-soluble product, and to ascertain if it were identical with the substance extracted by a dilute solution of sodium chloride from newly-made cheese. For the purpose of this comparison, the following experiment was undertaken to prepare artificially the salt-soluble product: About 6 liters of skim-milk were coagulated by rennet at 30° C. The resulting curd was well washed with water, drained, and then treated for one-half hour at 50° C. with 3 liters of a 1 per cent solution of lactic acid. By this treatment a turbid solution was obtained, which was filtered from the large residue remaining undissolved, through cloth first and then absorbent cotton. The filtrate, neutralized by dilute potassium hydroxide, gave a copious precipitate which was filtered, washed with water, and redissolved in 1 liter of a 0.5 per cent solution of lactic acid. This solution was filtered and reprecipitated by dilute alkali, and the process of redissolving, filtering, and reprecipitating was repeated twice. The resulting product was finally well washed

with water, suspended in 95 per cent alcohol for 2 days, and, after removal of alcohol by filtration, was extracted twenty-four hours with ether, and dried at 100° C. The substance thus prepared was firm and leathery in texture, greatly resembling in physical properties the product prepared from the salt extracts of cheese. To all appearances, the lactic acid had simply combined with the paracasein, forming a compound soluble in a slight excess of acid, and insoluble in neutral solutions.

(3) Preparation of Paracasein Lactate Obtained from Cheese.

—Cheese about three months old was extracted with a 5 per cent solution of sodium chloride. To 6.5 liters of this extract lactic acid was added to the extent of 0.2 per cent by volume, producing a flocculent precipitate that rapidly settled. The precipitate was filtered and allowed to drain, in order to remove the large amount of salt adhering. The filtrate gave no further precipitate on addition of more lactic acid. The precipitate was completely soluble in a 0.5 per cent solution of lactic acid, and this acid solution gave an abundant precipitate when neutralized by dilute alkali. This neutralization precipitate was filtered, well washed with water, and then treated, in every respect, as described above in the preparation of the artificial product. In this way 20 grams of each product were obtained.

(4) Comparison of Natural and Artificial Products.—The old method of comparing two products by the results of ultimate analysis, in order to establish their likeness or unlikeness, is now recognized as faulty, especially in the case of proteid bodies. Only by cleavage and by quantitative estimation of certain end-products can a reliable basis be found for determining the question of similarity of structure in different compounds.

Determinations of nitrogen, phosphorus, and ash were made in the two products. In addition, 10 grams of each proteid were hydrolyzed by boiling fourteen hours on a sand-bath, under a Liebig condenser, with 60 grams of water and 30 grams of sulphuric acid of sp. gr. 1.84. In the resulting

liquid, ammonia and the hexon bases were determined according to the method of Kossel and Kutscher.¹ The results are given in Table III., together with similar figures obtained by Hart² with casein prepared directly from milk by the use of acetic acid without rennet.

Table III.—Comparative Composition of Natural and Artificial Products.

	Nitrogen, expressed as percentage of total nitrogen, in form of				Percentage of		
	Ammonia.	Histidin.	Arginin.	Lysin.	Nitrogen.	Phosphorus.	Ash.
Natural product	7.65	2.36	7.77	2.21	15.12	0.70	1.51
Artificial "	7.66	2.21	8.08	2.02	15.30	0.91	1.120
Casein	7.34	3.66	9.51	2.31	15.65	0.84	

The results embodied in Table III. indicate a striking similarity in structure between the salt formed by paracasein with lactic acid, found naturally occurring in cheese, and that artificially prepared, so far as the products of hydrolysis enable us to draw conclusions. This has reference simply to the linking of the nitrogen in the proteid as a whole.

Later, it will be seen that what we had really prepared in both cases was the saturated salt formed by paracasein with lactic acid, and not the unsaturated salt; and, as we shall show, the unsaturated salt is the one soluble in a dilute solution of sodium chloride. The formation of these combinations with acids probably does not involve any deep-seated rupture of the proteid molecule, as can readily be inferred from the results given in Table III. with casein, which furnishes both saturated and unsaturated salts with acids. It is evident that in the preparation of the artificial salt, identical with the proteid extracted from cheese by a dilute solution of common salt, the chief, if not the only, active factor is an acid.

Salts Formed by Combination of Paracasein with Acids.

(1) *Salts Formed by Combination of Paracasein with Lactic Acid.*—In the manufacture of cheese, the lactic acid that com-

¹ Ztschr. physiol. Chem., 31, 165 (1898).

² *Ibid.*, 33, 347 (1900).

combines with paracasein is formed by the fermentation of milk-sugar, the lactic acid combining with the paracasein as rapidly as the acid is formed. It appeared desirable to study the action of lactic acid, used in different amounts, as it is formed in the fermentation process, upon paracasein under well-controlled conditions, and also to compare this action with the one taking place when pure, dilute lactic acid acts upon paracasein directly without any fermentation. For this purpose it was desired to secure paracasein or cheese-curd as free as possible from acid. In order to prevent all acid fermentation, very fresh, sweet milk was treated with ether. In this way it was possible nearly to eliminate the formation of paracasein compounds of lactic acid at the beginning of the experiment, and have as our original working material paracasein as nearly free as possible from salt-soluble compounds. Two analyses of paracasein, thus prepared, showed the presence of 3.90 and 4.34 per cent of the total nitrogen in the form of a salt-soluble compound.

Several bottles were prepared in the following manner: Twenty-five grams of the carefully prepared cheese-curd or paracasein, ground with sand, were placed in each bottle, 50 cc. of water were added, the mixture was thoroughly shaken, and then sterilized. To this common mixture various additions were made in the different bottles, as indicated below:

Series A received 0.5 gram of sterile milk-sugar and a pure culture of lactic acid organism.

Series B received 1.0 gram of sterile milk-sugar and the lactic acid organism.

Series C contained organism and no milk-sugar.

Series D received 0.5 gram of pure lactic acid and no organism.

Series E received 1.0 gram of pure lactic acid and no organism.

Series F received 1.5 grams of pure lactic acid and no organism.

All the bottles were kept at 15° C., and analyses were made at different periods, as indicated in Table IV.:

Table IV.—Influence of Different Amounts of Lactic Acid on Paracasein.

Series.	Grams of milk-sugar used.	Age of mixture when analyzed.	Presence of milk-sugar in mixture, when analyzed.	No. cc. N/10 NaOH required to neutralize 50 cc. of water extract.	Precipitate obtained after neutralizing water extract.	Nitrogen in form of salt-soluble product expressed as percentage of total nitrogen.
A	0.5	2 weeks	trace	3.9	yes	30.36
A	0.5	"	none	4.3	"	27.11
B	1.0	"	considerable	5.4	"	23.31
B	1.0	"	"	5.2	"	20.60
C	0	"	none	1.2	no	3.52
C	0	"	"	1.0	"	3.75
A	0.5	1 mo.	"	4.45	yes	40.65
A	0.5	"	"	3.9	"	28.46
B	1.0	"	little	5.2	"	9.76
B	1.0	"	"	4.9	"	7.32
C	0	"	none	0.6	no	3.52
C	0	"	"	0.8	"	4.07
Lactic acid.						
D	0.5	"	"	3.4	yes	44.72
E	1.0	3 mos.	"	6.4	"	2.17
F	1.5	1 mo.	"	11.4	"	1.62

Attention is called to certain facts shown by the data embodied in the table above.

1. When we used 0.5 gram of milk-sugar, the maximum amount of the product soluble in a dilute solution of sodium chloride was found only after the milk-sugar had completely disappeared, as can be seen by comparing series A at two weeks and one month.

2. When we used 1 gram of milk-sugar the largest amount of salt-soluble product was formed while considerable sugar was still present. As the acid fermentation of the milk-sugar continued, the amount of salt-soluble product rapidly decreased. This is shown by comparing Series B at two weeks and one month.

3. We see the same truth illustrated in Series D, E, and F,

where we added pure lactic acid directly in the different quantities stated. When we added 0.5 gram of lactic acid there was formed a large amount of salt-soluble product, nearly 45 per cent of the total nitrogen appearing in this form. However, when we used 1 and 1.5 grams of pure lactic acid there was practically no salt-soluble product formed.

4. Even in the cases where the largest amounts of lactic acid were used the acid disappeared as free acid. This was shown by making a titration of the water extract of the curd with N/10 sodium hydroxide, using phenolphthalein as indicator. It is readily seen that the number of cubic centimeters of alkali required for neutralization represents a very small amount of acid, compared with that furnished in Series A, B, D, E, and F. The acid had largely combined with paracasein to form products only slightly soluble in water.

The question may be raised here as to what it is in the water extract that neutralizes alkali. In Series C it is, in all probability, direct neutralization by proteid, as no acid was present. In Series F, on the other hand, there is probably some free acid, as an excess of acid was used. In most cases, however, the neutralization is due, apparently, to a water-soluble compound of acid and proteid, as shown by the fact that only in cases where acid was furnished, whether by fermentation or by direct addition, a precipitate appeared on neutralization, and such a precipitate would be expected only in the case of a combination of acid with proteid. The larger the amount of alkali required by the water extract for neutralization, the more abundant was the precipitate appearing on neutralization.

Just here, the main point we desire to consider is an explanation of the fact to which attention has been called above, *viz.*, that from a given amount of paracasein, treated with a certain amount of lactic acid, we obtain a maximum yield of our salt-soluble product, while an increase of acid beyond a given amount decreases the yield of salt-soluble product. Naturally, we might expect that increase of acid would yield an increase of salt-soluble product from a given quantity of

paracasein. How can we explain this? The explanation that agrees most satisfactorily with the experimental data at hand is this: Paracasein combines with lactic acid in at least two different proportions, forming two distinct compounds. One of these compounds is the unsaturated salt and is soluble in dilute solutions of sodium chloride. It is this compound that is present in normal cheddar cheese. The other compound is the saturated salt formed by the combination of paracasein with lactic acid and is insoluble in a dilute solution of salt.

(2) *Salts Formed by the Combination of Paracasein with Other Acids.*—Paracasein forms salts also with other acids.

Mr. A. J. Patten has determined for us the amounts of acetic, hydrochloric, and sulphuric acids, required to form the unsaturated and saturated salts. In each case acid was added to 25 grams of fresh curd, previously ground with sand, suspended in water, and finally extracted at 55° C. with a 5 per cent solution of sodium chloride.

Table V.—Formation of Compounds by Paracasein and Different Acids.

	Acetic acid.		Hydrochloric acid.			Sulphuric acid.		
Grams of acid used	0.5	1.0	0.15	0.25	0.50	0.15	0.25	0.50
Percentage of total nitrogen found in form of salt-soluble compound	70.0	5.3	27.3	53.0	5.7	19.5	41.5	2.4

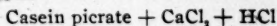
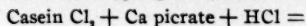
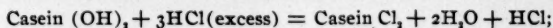
In this table the same general results are seen, as in the case of lactic acid. A certain amount of acid forms with a given amount of paracasein a maximum quantity of salt-soluble product, and increase of acid beyond this quantity forms a compound not soluble in a dilute solution of salt.

It also appears from the results given in Table V. that mineral acids are required in smaller amounts than organic acids to saturate paracasein completely.

Salts Formed by Combination of Casein with Acids.

(1) *Formation of the Unsaturated Casein Salt.*—We can show that casein readily forms an unsaturated and a saturated salt with an acid. In preparing the unsaturated salt we used 5 cc. of freshly-drawn milk, diluted with 50 cc. of water, warmed the solution to 40° C., and then added N/10 hydrochloric acid until the resulting precipitate settled leaving a clear supernatant liquid, which required 2.7 cc. To this mixture were added 2.5 grams of sodium chloride, thus making a 5 per cent salt solution. On warming, the unsaturated casein chloride completely dissolved. The experiment was repeated several times with a uniform result. On adding more acid to the salt solution the proteid reprecipitated as the saturated chloride of casein. Casein behaved toward acetic acid in the same manner.

(2) *Determination of Amount of N/10 Hydrochloric Acid Required to Saturate Casein.*—Fresh milk was drawn from a cow directly into a bottle containing ether, thus checking all acid fermentation. Of this milk 5 cc., containing 0.0261 gram of nitrogen, were diluted with 50 cc. of water that had been boiled free from all carbon dioxide. The mixture was warmed to 40° C. and 10 cc. of N/10 hydrochloric acid were added. It was desired now to determine the amount of hydrochloric acid that had combined with casein. Determinations of the uncombined acid did not give uniform results, when the titration was made directly after the addition of acid. We therefore employed the method of using calcium picrate.¹ There were added 5 cc. of a neutral solution of calcium picrate to precipitate all proteids completely. The precipitate was filtered off and the filtrate treated with N/10 sodium hydroxide, using rosolic acid as indicator. The reactions taking place can be represented, theoretically, as follows:



¹ Rohrer: *Archiv. für Physiol.*, 90, 368 (1902).

When calcium picrate is used, the chlorine which was combined with casein, is changed into calcium chloride, and in this form does not interfere with titration by alkali. Thus we are able to determine more satisfactorily the amount of acid left uncombined with proteid.

In the experiment employed it required 7.4 cc. N/10 sodium hydroxide to neutralize the excess of acid, indicating that 2.6 cc. of acid had entered into combination with 0.0261 gram of proteid nitrogen. Using 6.39 as the nitrogen factor of casein, 0.0261 gram of nitrogen is equivalent to 0.167 gram of casein, and this amount of casein combined with 2.6 cc. N/10 hydrochloric acid, or 1 gram of casein would require for complete saturation 15.6 cc. of N/10 hydrochloric acid. The work was repeated on 5 cc. of milk containing 0.031 gram of nitrogen, and gave 15.9 cc. of N/10 hydrochloric acid as the amount required to saturate 1 gram of casein.

In the manner in which this work was done it is recognized that we were dealing not with casein alone but with the two principal proteids of milk, casein and albumin. However, there is no reason to believe that the results would be essentially different with pure casein. We shall later secure results with casein alone.

(3) *Determination of Amount of N/10 Hydrochloric Acid Required to Saturate the Unsaturated Salt of Paracasein.*—To 50 cc. of the salt extract of some curd, containing 0.058 gram of nitrogen in the form of the unsaturated hydrochloric acid salt of paracasein, were added 10 cc. of N/10 hydrochloric acid for the purpose of converting the unsaturated into the saturated salt. There were then added 5 cc. of neutral calcium picrate to facilitate clear filtration. The mixture was filtered, the precipitate washed, and the filtrate treated with N/10 sodium hydroxide, rosolic acid being used as indicator. Neutralization required 7.1 cc. of N/10 sodium hydroxide. Hence, to saturate the salt-soluble compound, equivalent to 0.058 gram of nitrogen, required 2.9 cc. N/10 hydrochloric acid. Using 6.39 as the nitrogen factor of paracasein, it required 7.83 cc.

of N/10 hydrochloric acid to convert 1 gram of the unsaturated into the saturated hydrochloric acid salt of paracasein.

We saw above that it required from 15.6 to 15.9 cc. of N/10 hydrochloric acid to saturate milk-casein completely, or, more strictly, casein mixed with a small amount of milk-albumin. Now, the amount of N/10 hydrochloric acid required to convert the unsaturated into the saturated hydrochloric acid salt of paracasein, 7.83 cc., is just one-half of 15.7 cc., the amount of N/10 hydrochloric acid required to form a saturated compound. Hence, the amount of combined acid in the saturated salt is twice that in the unsaturated salt; in other words, the unsaturated salt appears to be a mono-acid salt, and the saturated a di-acid salt. These results, it is true, were obtained, in one case, with a milk-casein not strictly pure, and, in the other, with the paracasein derivative of milk-casein; and it may be thought that results secured in this way do not justify the conclusion drawn in regard to the existence of two such distinct combinations of an acid with casein and paracasein. But, aside from the data presented here, we have abundant evidence that both milk-casein and paracasein form at least two salts, corresponding to a half-saturated or mono-acid salt, and a saturated or di-acid salt.

Possibility of an Additional Compound of Paracasein with Acid.

There is evidence that small amounts of acids can disappear in cheese-curd without a corresponding increase of salt-soluble compound. This fact is illustrated by the following experiment: Three liters of freshly-drawn milk, to which ether had been promptly added, were coagulated with rennet and then warmed to 42° C. in order to shrink and harden the curd. Portions of 25 grams each of this curd were ground with sand, placed in Erlenmeyer flasks, covered with 100 cc. of water, heated for ten minutes at 85° C. to check completely all acid fermentation, and then treated with different amounts of lactic acid. After standing one hour, with occasional shaking, the curd in the different flasks was extracted, first with small amounts of water and then with 500 cc. of a 5 per

cent solution of sodium chloride. In 50 cc. of each of these extracts the nitrogen was determined by the Kjeldahl method. The results are given in the following table.

Table VI.—Amounts of Salt-soluble Compound Formed by Different Amounts of Acid.

Grams of lactic acid used	0	0.02	0.05	0.10	0.20	0.50
Percentage of total nitrogen in form of salt-soluble compounds	6.0	7.0	8.1	8.6	23.0	72.0

The amount of salt-soluble compounds was not proportionally increased by the use of 0.02, 0.05, and 0.10 gram of lactic acid for 25 grams of curd, but when an additional 0.10 gram of lactic acid was used, bringing the total up to 0.20 gram of lactic acid, there was a large increase of the salt-soluble compound, and this was still greatly increased by additions of lactic acid above 0.20 gram up to 0.50 gram. It is possible that there may be formed with acids a paracasein salt containing still less acid than the half-saturated salt, or, it may be, that the acid which disappears unites with some of the insoluble inorganic salts in the curd.

Solubility of the Salts Formed by Casein and Paracasein with Acids.

(1) *Saturated Salts.*—The saturated acetic and lactic acid salts of casein and paracasein, as well as the hydrochloric and sulphuric acid salts, are practically insoluble in all the media tried. They are insoluble in water, in 5 and 10 per cent solutions of sodium chloride, slightly soluble in 50 per cent boiling alcohol, practically insoluble at 50° C. in saturated solution of calcium carbonate, and in 2 per cent solution of calcium lactate. They dissolve, of course, in excess of acid or dilute alkali.

(2) *Unsaturated Salts.*—The unsaturated or half-saturated salt of each of the acids mentioned above is insoluble in water, completely soluble in 5 per cent solution of sodium chloride at 55° or 60° C., not separating again on cooling, but entirely separating by dialysis in water; soluble in 50 per cent boil-

ing alcohol, but separating again on cooling; only slightly soluble at 50° C. in saturated solution of calcium carbonate, or in 2 per cent solution of calcium lactate.

(3) *Explanation of Danilewsky's Error.*—Danilewsky's¹ early supposition that casein consists of two proteid bodies separable by hot 50 per cent alcohol, finds a reasonable explanation in the fact that the unsaturated salt formed by casein with an acid is soluble in 50 per cent hot alcohol, while the saturated salt is insoluble in this medium. When the usual method is employed of precipitating casein from milk by means of dilute acid, the resulting product can easily be a mixture of the unsaturated and saturated salts, the quantity of each present depending upon the amount of acid used in the precipitation. Danilewsky, in his work, used only enough hydrochloric acid to effect a good precipitation of milk-casein, and thus he formed more or less of the unsaturated salt, soluble in 50 per cent hot alcohol. Thus it is easy to see how he came to the erroneous conclusion that milk-casein consists of two proteids.

(4) *Weidmann's Caseoglutin.*—Referring again to Weidmann's caseoglutin, dissolved from cheese by hot alcohol, there appears to be every reason to believe that it is identical with the unsaturated paracasein lactate described in this paper.

(5) *Relation of Salts to Freudenreich's Results.*—It will be noticed that the solubility of the two salts in a solution of calcium carbonate or calcium lactate is slight. In the case of the saturated paracasein salt, it was found in one experiment that only 1.2 per cent of the salt was soluble at 50° C. in a saturated solution of calcium carbonate, and only 1.12 per cent was soluble in a 2 per cent solution of calcium lactate at 50° C. The unsaturated paracasein salt had a solubility of 5.01 per cent in the solution of calcium carbonate, and 4.70 per cent in the solution of calcium lactate.

The determination of these solubilities was made in the hope of finding some explanation of the results obtained by Freud-

¹ Ztschr. physiol. Chem., 7, 227 (1883).

enreich¹ in his studies relating to the power of lactic-acid organisms to effect proteolytic changes in milk-casein. Freudenreich found that sterile milk, treated with powdered calcium carbonate and then seeded with certain bacteria that produce lactic acid, showed an increase of soluble nitrogen compounds, in both caseoses and amides. He determined the soluble nitrogen by passing the milk through a Chamberland filter. He does not state the amount of total nitrogen in the milk at the beginning of the experiment, but only the amount of soluble nitrogen. Assuming that the milk used by Freudenreich contained 0.5 per cent of total nitrogen, the average of milk, and recalculating his results in percentages of total nitrogen, we obtain the data given in the following table:

Table VII.—Results of Freudenreich Calculated on Basis of Total Nitrogen in Milk.

Age of milk when analyzed.	Soluble nitrogen, expressed as percentage of total nitrogen in milk.	Amide nitrogen, expressed as percentage of total nitrogen in milk.	Organism used.
Begin'ng of exp't	6.6		
9 months	47.0	34.6	Milk culture, Bac. ϵ from rennet
13 "	44.4	30.2	" " " " " cheese
13 "	49.2		" " " " " "
14 "	42.8	28.0	Bac. α and bac. δ
9 "	18.8	10.6	" α

The amounts of soluble nitrogen reported by Freudenreich as being formed by the action of different organisms that produce lactic acid are considerable. Moreover, it is to be noticed that quite large amounts of amide nitrogen were formed. While there were in the flasks used dilute solutions of calcium carbonate and of calcium lactate, we cannot explain the relatively large amounts of soluble nitrogen by the solubility of either the saturated or unsaturated lactates of casein in the solution of calcium carbonate or lactate. Whether his explanation is correct or another is to be found,

¹ Landw. Jahr. d. Schweiz., 12, 279 (1895).

Freudenreich's results clearly represent actual proteolytic changes of casein.

Nicholson¹ repeated Freudenreich's work, using the common lactic-acid bacterium found in cheese, but failed to find any proteolysis of casein. Nicholson says: "In some cases, the table shows a slight increase in the soluble nitrogen, where the calcium carbonate has been added; in others there is no increase. In the uncarbonated milk there is absolutely no increase in any case." The slight increase reported by Nicholson in the case of the milk containing calcium carbonate can readily be explained on the basis of the slight solubility of the lactic acid salts of casein in solution of calcium carbonate or calcium lactate.

We have recalculated a portion of Nicholson's results on the same basis as that used with Freudenreich's figures, and give them in the table following:

Table VIII.—Results of Nicholson's Work with Lactic-acid Bacteria.

Without calcium carbonate.			With calcium carbonate.		
Age of milk when analyzed.	Soluble nitrogen, expressed as percentage of total nitrogen in milk.	Amide nitrogen, expressed as percentage of total nitrogen in milk.	Age of milk when analyzed.	Soluble nitrogen, expressed as percentage of total nitrogen in milk.	Amide nitrogen, expressed as percentage of total nitrogen in milk.
0	8.8	3.8	0	9.6	4.0
0	9.2	3.6	0	9.8	4.0
59 days	13.2	3.2	28 days	18.0	4.0
59 "	12.6	3.0	28 "	21.8	2.8
450 "	10.2	1.4	257 "	12.6	2.2
450 "	8.4	trace	257 "	12.2	trace
			314 "	13.2	2.8
			314 "	12.0	3.2
			450 "	12.6	trace
			450 "	14.0	"

In some of our future work, bearing more directly upon the chemical changes taking place during the process of ripening

¹ Thesis for Master's degree, Univ. of Wis., 1902.

in cheese, we shall have occasion to refer to this subject again.

Relation of the Unsaturated Paracasein Lactate to the Cheddar Process of Cheese-making.

As previously stated, it has been supposed that the presence of lactic acid was, in some way or other, responsible for, or associated with, some of the most important changes taking place in the curd during the cheese-making process, such as the acquired ability to form strings on hot iron and the change in the appearance and plasticity of curd, but no one has previously been able to show beyond question that these changes are actually brought about by an acid, or in what way they are accomplished. We purpose now to show the intimate relation existing between the presence of unsaturated paracasein lactate and some of the important changes occurring in the curd during the process of making cheese.

(1) *Property of Curd to String on Hot Iron Due to Unsaturated Paracasein Lactate.*—About 27 liters of milk, properly ripened for cheese-making, were coagulated by rennet, and the process of treatment was carried on in the usual normal manner employed in manufacture. At different intervals samples of curd were taken in order to determine the amount of salt-soluble compound present; also samples of whey were taken at the same time for the purpose of determining the amount of milk-sugar. The changes took place rather slowly in this instance, owing to the fact that the milk was more than usually sweet for cheese-making purposes, that is, it was so fresh as to contain only small amounts of lactic acid at the start. We present our results below in tabular form:

Table IX.—Increase of Salt-soluble Compound in Curd with Decrease of Milk-sugar in Whey.

Time of taking sample of curd,	Percentage of total nitrogen in form of salt-soluble compound.	Percentage of milk sugar in whey.	Remarks.
Rennet added at 9.30 o'clock A.M.			
10 o'clock A.M.	5.0	4.75	Curd did not string.
12 " M.	6.1	...	Curd just began to string. Whey removed from curd.
4 " P.M.	31.7	1.83	Curd formed strings 1 inch long. Curd put in press.

The results in this table show that, as the amount of salt-soluble compound present in the curd increased, the curd acquired the property of forming longer strings on the hot iron. That this was due to the increase of salt-soluble compound is confirmed by the fact that the unsaturated salt of casein lactate itself, when isolated from curd or cheese, forms on hot iron beautiful, fine, silky, threads, of almost any length desired. The same is true also of the product artificially prepared.

(2) "*Breaking-down*" Process in Curd Due to Formation of Unsaturated Paracasein Lactate.—After the whey is removed from the curd in the process of cheese-making, the curd is "packed" or "matted," that is, piled in a heap and kept in this condition, with occasional repacking, until it has gone through the regular "breaking-down" process, in the course of which the curd undergoes several marked, easily discernible changes in physical properties. From a tough, rubber-like consistency, with a high water-content, the curd changes to a mass of lower water-content, having a smooth, velvety appearance and feeling, and a softer, somewhat plastic consistency. The texture also changes so that the curd acquires a peculiar kind of grain and tears off somewhat like the cooked meat of a chicken's breast.

These marked changes in the physical properties of the curd can be most readily and satisfactorily explained by attributing them to the increasing quantity of the unsaturated casein lactate produced throughout the cheese mass as the result of the continuous formation of lactic acid by the fermentation of the milk-sugar present. These changes in the properties of the curd take place simultaneously with the formation of unsaturated casein lactate and do not take place in the absence of this compound. The softening of curd, or "breaking-down" process, is attributed by Duclaux to the action of *Vibrios*, but this is certainly an error so far as related to American cheddar cheese.

(3) *Abnormal Softening of Curd on Addition of Salt, Due to the Presence of the Unsaturated Paracasein Lactate.*—It sometimes happens that, when curd is salted, it softens remarkably, appears to be slimy, and slips through the fingers when squeezed in the hand. This is a condition which has not been satisfactorily explained. The usual explanation is that liquefying organisms have prevailed over all others and have dissolved some of the curd. The real explanation is as follows: When curd behaves, on salting, in the manner described above, more than the usual amount of lactic acid has been formed, and, hence, more than the usual proportion of unsaturated paracasein lactate has resulted. When salt is added to such curd, we at once have a brine formed, which is capable of dissolving the unsaturated paracasein lactate contained in the curd, thus producing a pasty, soft, slippery mass.

In an experiment in which we made cheese from pasteurized milk, we endeavored to simulate the action of lactic acid, as formed by fermentation in ordinary cheese-making, by substituting in its place hydrochloric acid, added in small quantities from time to time, both before and after the addition of rennet. Paracasein chloride was readily formed by the curd, but the use of somewhat too much acid produced an abnormally large proportion of the unsaturated salt, which, on addition of common salt, formed a mushy, slippery mass. The curd, in this condition, was suspended in water and more

hydrochloric acid was added, resulting in the production of a firm, exceedingly hard curd, due to the conversion of unsaturated into saturated paracasein chloride, the latter compound not being soluble in salt solution.

(4) *Amount of Unsaturated Paracasein Lactate in Newly-made Cheese.*—In new cheese, fresh from the press, prepared under normal conditions, we have found as much as 78.5 per cent of the nitrogen compounds present in the form of unsaturated paracasein lactate. The proportion of this compound in new cheese depends upon a variety of conditions which we are engaged in studying. It appears probable that in the case of normal, newly-made cheese the nitrogen compounds consist mainly of unchanged paracasein and unsaturated paracasein lactate, the latter compound being present in larger proportions. Saturated paracasein lactate probably does not occur in normal cheese, but only in cheese made with excessive acid, as, for instance, in the case of cheese made from sour milk.

Relation of Unsaturated Paracasein Lactate to Cheese-ripening.

As cheese ages it undergoes various changes, passing from an indigestible, insoluble, and flavorless substance into the palatable, nutritious material we use as food. The term "ripening" is applied to the general process that embraces these changes. Among the most extensive and profound changes occurring in cheese-ripening are those experienced by the nitrogen compounds of the newly-made cheese, chief of which is unsaturated paracasein lactate.

A large amount of bacteriological and chemical work has been done in order to ascertain the cause of the changes that occur in the nitrogen compounds during the ripening of cheese. All of this work has, without exception, assumed that paracasein formed the primary point of attack or the starting-point in these changes. Our results, presented in the foregoing pages, suggest that the unsaturated paracasein lactate, and not paracasein, is the real material with which the process of cheese-ripening commences. It is not our purpose,

at this time, to go into the subject in more than a preliminary way. In connection with Table II., p. 417, attention has been called to the fact that the amount of water-soluble nitrogen in cheese, which is a general measure of the extent of cheese-ripening, increases at the same time the unsaturated paracasein lactate decreases, and probably at the expense of the latter compound.

The belief that the proteolytic changes of cheese-ripening actually start with the unsaturated paracasein lactate, receives confirmatory evidence from the fact that, in the absence of unsaturated paracasein lactate in cheese, we have very incomplete proteolytic change. In order to establish beyond question the specific relation of unsaturated paracasein lactate to the proteolytic changes of cheese-ripening, we have under way numerous experiments in which preparations of pure, unsaturated paracasein lactate are being subjected to the action of a variety of agencies, such as different enzymes and organisms that have been commonly associated with the different theories of cheese-ripening.

Some results already secured appear to indicate that the first proteolytic changes in cheese-ripening are the result of peptic digestion of unsaturated paracasein lactate. In cheese, pepsin appears to act, not to any marked extent upon the proteid paracasein, but only upon its unsaturated compounds with acids. Hence, the function of an acid in cheese-making is the production of the unsaturated compound of paracasein with acid. This suggests that somewhat the same kind of action may be found to be true in the case of gastric digestion. It is well established that pepsin acts upon proteids only in the presence of dilute acid. The function of the hydrochloric acid of gastric juice, like lactic acid in cheese, may be the formation of compounds with proteids; in other words, it may be found that pepsin in gastric digestion does not act upon pure proteids but only upon their compounds with acids, in this case hydrochloric acid. While this general view has been held for some time there have not, so far as we know, been any actual experiments previously made in test-

ing the action of pepsin upon pure compounds formed by proteids with acids. We have done this only in the case of the unsaturated paracasein lactate, but this appears as the first instance to furnish proof in an individual case of what may be found true, in general, of all cases of peptic digestion.

Summary.

1. The object of the work described in this paper is to study the real function of acids in relation to the important changes taking place in cheese curd during the cheddar process of cheese-making.

2. In examining cheese for heterocaseose, by extraction with a dilute solution of common salt, a body was found in quantities so large as to indicate that it was some compound other than heterocaseose.

3. In studying cheeses made with and without the use of lactic acid, the salt-soluble product was discovered to be present in very large quantities only when acid was used, being practically absent, or present in very small proportions, when no acid was used.

4. In normal cheese the salt-soluble product is always found, but in varying quantities, being largest usually in new cheese and diminishing as the cheese ages. Various conditions affect the amount in new cheese, especially those conditions influencing the amount of acid present.

5. Paracasein, carefully prepared and treated with dilute lactic acid, furnishes a product resembling in both physical and chemical properties the salt-soluble substance extracted from cheese.

6. Paracasein is shown to combine with an acid in at least two different proportions, forming two distinct compounds: one is the unsaturated or mono-acid salt; the other is the saturated or di-acid salt. Such compounds were prepared with lactic, acetic, hydrochloric, and sulphuric acids.

7. Casein forms salts with acids in the same manner as paracasein.

8. The unsaturated salts formed by casein and paracasein with acids are soluble in dilute solutions of sodium chloride and in hot 50 per cent alcohol, but are insoluble in water. The saturated salts are practically insoluble in water, dilute salt solutions, and hot 50 per cent alcohol. Both forms are sparingly soluble in dilute solutions of calcium carbonate and calcium lactate.

9. The important changes taking place in cheese-curd during the process of cheddar cheese-making, such as the acquired ability to form strings on hot iron, the changes in appearance, plasticity, and texture, and probably the shrinking, are due to the formation of the unsaturated paracasein lactate.

10. The ripening process in normal cheddar cheese, by which the insoluble nitrogen compounds change into soluble forms, begins not with paracasein, as has been universally held, but with unsaturated paracasein lactate. The water-soluble nitrogen in cheese generally increases as the unsaturated paracasein lactate decreases, and apparently at the expense of the latter compound. The first step in the normal ripening process of American cheddar cheese is probably a peptic digestion of unsaturated paracasein lactate. Some of the facts presented suggest a method of proof of the commonly accepted theory of gastric digestion.

GENEVA, N. Y., July 31, 1902.

Contribution from the Kent Chemical Laboratory of Yale University.

ON THE RELATION OF HYDRIODIC ACID AND OF ITS SALTS TO THE STARCH AND DEX-TRIN IODIDES.

BY F. E. HALE.

There has been much disputation and uncertainty about the presence of hydriodic acid, or its salts, in starch iodide. Mylius,¹ in 1887, found hydriodic acid and iodine present in starch iodide in the ratio of 1 molecule of hydriodic acid to 4 atoms of iodine, by the treatment of filtrates from starch iodide

¹ Ber. d. chem. Ges., 20, 688-695.

precipitated in the presence of known amounts of iodine and potassium iodide. Lonnes,¹ in 1894, corroborated Mylius' work by a similar method and found the ratio HI : 4I. Lonnes also analyzed the filtered and washed starch iodide and found a different ratio, HI : 3I, for which fact he offered no explanation, though he mentioned that there always appeared in his wash-water free iodine which he estimated by thiosulphate. As the influence of potassium iodide upon the starch iodide reaction is so marked it seemed proper to investigate again the amount of hydriodic acid, if any, present in starch iodide. After many failures with unsatisfactory methods the following method was devised and carefully carried through with results closer than those of Mylius and Lonnes, and corroborating the ratio HI : 4I for starch iodide.

The starch solution was made from pure potato starch which had been washed with cold water and dried in a vacuum desiccator over sulphuric acid. The consistency of the starch solution was that of a thin paste. As no cellulose settled out and the solution could not be filtered, it was used just as it was. It was made up approximately in 0.5 per cent solution.

The principle of the first and most exact method of analysis consisted in precipitating completely as the starch iodide by an excess of starch solution the free iodine of an exactly measured amount of iodine solution, made up as usual with potassium iodide, and determining the amount of potassium iodide removed from the iodine solution by the starch iodide. The details are as follows :

Into a 500 cc. standard flask 200 cc. of a 0.5 per cent starch solution (the amount determined by experiment) and 10 cc. of dilute sulphuric acid (1 : 3) were introduced, and 40 cc. of N/100 iodine solution were run in from a burette. The mixture was thoroughly shaken, diluted to the mark, shaken again, and allowed to settle. In three to twenty-four hours the starch iodide settled to the bottom of the flask. By means of a suction-pump the supernatant colorless liquid was drawn off into a dry flask, and then was filtered through a dry filter-paper to remove small amounts of starch iodide. Of this

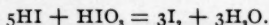
¹ *Ztschr. anal. Chem.*, 1894, p. 409.

liquid, 250 cc. were drawn from a 100 cc. burette into a Drexel wash-bottle (Fig. 1.) fitted with a ground-glass stopper carrying the usual inlet and outlet tubes. To the inlet tube had been sealed a separating-funnel to serve for the introduction



of reagents, and to the outlet tube a Will and Varrentrapp bulb-tube charged with a fairly concentrated solution of potassium iodide to catch the iodine carried off mechanically by escaping carbon dioxide during the neutralization of the acid by acid potassium carbonate. Through the separating-funnel 5 cc. of a 0.5 per cent iodic acid solution were added, the proper amount of sulphuric acid being already present in the solution

since sulphuric acid had been used to precipitate the starch iodide, and the mixture thoroughly shaken and allowed to stand for five to fifteen minutes. Iodic acid in excess liberates all the iodine of hydriodic acid under proper conditions, as worked out by Gooch and Walker¹ in this laboratory, according to the equation



Therefore, five-sixths of the iodine set free represents the hydriodic acid present. The solution was then neutralized, gradually and with thorough shaking, and made alkaline by means of about 50 cc. of a saturated solution of acid potassium carbonate, 50 cc. of N/100 arsenite introduced, the contents of the trap added, the trap thoroughly washed, and the excess of arsenite titrated by N/100 iodine, with starch as an indicator. The standard of the N/100 iodine was determined by the N/100 arsenite at 500 cc. volume and in the presence of the same amount of potassium iodide as was introduced into the trap to catch escaping iodine. Five-sixths of the difference between the standard for 50 cc. of N/100 arsenite in terms of N/100 iodine and the amount of N/100 iodine necessary to titrate the excess of arsenite above, represents the amount of iodine corresponding to the amount of potassium iodide left in the iodine solution by the starch iodide. As 40 cc. of

¹ Am. J. Sci., 1897, p. 293.

N/100 iodine were taken, diluted to 500 cc., and 250 cc. of this solution used, the amount of potassium iodide determined was that left in 20 cc. of the N/100 iodine solution. Hence the potassium iodide originally present in 20 cc. of the N/100 iodine solution had to be determined in a similar manner. Into the trapped bottle 20 cc. of the N/100 iodine solution were drawn, 5 cc. of iodic acid and 5 cc. of dilute sulphuric acid (1 : 3) introduced, the mixture shaken after introducing potassium iodide into the trap, and the analysis proceeded with as in the previous case, except that from the total iodine liberated the free iodine of the 20 cc. of N/100 iodine had to be subtracted before finding five-sixths. The difference between the amount of potassium iodide originally present and the amount left there by the starch iodide is the amount used in forming starch iodide.

Analyses.

Standardization.—In the presence of 25 cc. of acid potassium carbonate, 10 cc. of potassium iodide solution, and 3 to 5 cc. of pure starch solution in a total volume of 500 cc., 50 cc. of N/100 arsenite required 50 cc. of N/100 iodine to bring a pale-blue color in two duplicates.

Determination of Potassium Iodide in 20 cc. of N/100 Iodine Solution.

	I. N/100 iodine solution. cc.	II. N/100 iodine solution. cc.
50 cc. N/100 arsenite solution	50.00	50.00
Excess of arsenite solution	5.30	5.20
Free iodine of 20 cc. N/100 iodine and total iodine set free by iodic acid	44.70	44.80
Free iodine of 20 cc. N/100 iodine sol.	20.00	20.00
Iodine set free by iodic acid	24.70	24.80
$\frac{5}{8} \times I$ by iodic acid = I of potassium iodide in 20 cc. N/100 iodine solution	20.58	20.67
Average—20.63 cc. N/100 iodine = iodine of potassium iodide in 20 cc. of N/100 iodine.		

Determination of the Ratio of Iodine to Potassium Iodide in Starch Iodide.

	N/100 iodine. cc.
50 cc. N/100 arsenite solution	50.00
Excess of arsenite solution	31.24
Iodine set free by iodic acid	18.76
$\frac{3}{8} \times 18.76 =$ iodine of potassium iodide left in 20 cc. N/100 iodine	15.63
Iodine of potassium iodide in 20 cc. N/100 iodine	20.63
Iodine of potassium iodide left in 20 cc. N/100 iodine	15.63

Iodine of potassium iodide in starch iodide 5.00

But 20.00 cc. N/100 iodine = iodine of starch iodide; therefore, the ratio of potassium iodide to iodine in starch iodide is 1 : 4.

In five other analyses the excess of arsenite was found to be represented by the following amounts of N/100 iodine: 31.20 cc., 31.39 cc., 31.38 cc., 31.14 cc., 31.24 cc., which give the ratio of potassium iodide to iodine as 1 : 4.03, 1 : 3.91, 1 : 3.91, 1 : 4.07, 1 : 4.

These analyses, which point to an average ratio of 1 : 3.99, are so close that they seem to prove absolutely the presence and amount of hydriodic acid in starch iodide.

An attempt was now made to analyze the starch iodide itself. The principle of the method consists in treating the washed starch iodide with an excess of arsenite, titrating the excess by iodine (thus determining the amount of iodine in the starch iodide), determining the total amount of potassium iodide now present and the amounts introduced during the analysis, and so by difference the amount of potassium iodide in the starch iodide. The details are as follows :

The precipitates of the previous experiments were filtered by means of ordinary filter-paper, drained, and washed by filling the funnel four to five times with dilute sulphuric acid (10 cc. of 1 : 3 acid diluted to 500 cc.), and allowing to drain almost or quite dry. A few drops of the final wash-water

gave no test for iodine on adding iodic acid and shaking with chloroform. The precipitates were transferred to beakers by scraping with a glass rod and directing a strong stream of water against them from a wash-bottle, and allowed to settle. The supernatant water was poured off and 50 cc. of N/100 arsenite and 25 cc. of acid potassium carbonate were added to each beaker. In twenty-four hours to a week all the iodine had been taken up. The excess of arsenite was then titrated and gave, by difference, the amount of iodine. The beakers were nearly filled with alcohol (total volume about 400 cc.), and thoroughly stirred to precipitate the starch, as it was found that iodic acid did not free all the iodine from the hydriodic acid in the presence of so much starch. The starch was filtered on asbestos in a Gooch crucible, and quantitatively determined in one case. Before filtering it was absolutely necessary to let the starch settle, and during filtration to keep water out of contact with the starch, else the starch became pasty and clogged the filter. Washing with absolute alcohol every little while was necessary to keep the filter open. The starch was finally washed with ether and a Bunsen flame fanned beneath the crucible until the odor of ether was imperceptible. The crucible was allowed to stand in a sulphuric acid desiccator and weighed. It was then ignited until all starch was burned out, and weighed again. The difference gave the amount of starch. The filtrates were evaporated to dryness on the steam-bath to get rid of the alcohol and ether, the residue taken up with a little water, washed into the Drexel wash-bottle, and the total potassium iodide determined as before, except for the amounts of reagents used. This amount of potassium iodide represents three distinct amounts, the potassium iodide of the starch iodide, the potassium iodide formed from the free iodine of the starch iodide and of the iodine solution introduced, and the potassium iodide always present in the iodine solution introduced. The amount of potassium iodide in 20 cc. of N/100 iodine was determined in the previous experiments and so could be calculated for any amount. The amount of potassium iodide formed from free iodine is converted back into free iodine and

is represented by the iodine value for 50 cc. of N/100 arsenite. The amount of potassium iodide in the starch iodide is found by subtracting the two latter amounts from the total.

Analyses.

	I. N/100 iodine. cc.	II. N/100 iodine. cc.
50 cc. N/100 arsenite	50.00	50.00
Excess of arsenite solution	30.00	24.70
Iodine of starch iodide	20.00	25.30
110 cc. and 105 cc. N/100 arsenite	110.00	105.00
Excess of arsenite solution	5.68	4.60
Total iodine set free by iodic acid	104.32	100.40
$\frac{5}{8} \times 104.32$ and $100.40 =$ iodine of potassium iodide freed by iodic acid	86.93	83.67
50 cc. N/100 arsenite	50.00	50.00
Iodine of potassium iodide in iodine solution introduced and in starch iodide	36.93	33.67
Iodine of potassium iodide in iodine solution introduced	31.46	25.58
Iodine of potassium iodide in starch iodide	5.47	8.09
Ratio of potassium iodide to iodine	$\left\{ \begin{array}{l} 5.47 : 20 \quad 8.09 : 25.30 \\ 1 : 3.66 \quad 1 : 3.13 \end{array} \right.$	

Another analysis of a smaller amount, made by a similar method, but without precipitating the starch, gave a result of 1.67 : 5.12. The ratio is 1 : 3.07.

While the amount of potassium iodide in these analyses is somewhat increased, yet the results are so irregular that they seem to be only roughly corroborative of the ratio obtained by the more exact method. It is more than probable that this increased ratio is due to the difficulty of washing out the last traces of included potassium iodide from the starch iodide.

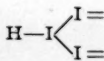
The Red Starch Iodide.—The influence of concentrated potassium iodide upon the color of starch iodide has been noted by many writers. Mylius,¹ in 1895, noted the power of

¹ Ber. d. chem. Ges., 28, 385.

water to destroy the red color produced by iodine and concentrated potassium iodide, and suggested that the red starch iodide contained no potassium iodide, but twice as much iodine as the blue starch iodide, performing, however, no experiments to prove his idea. On account of the fact that concentrated potassium iodide turns starch iodide blue to starch iodide red, that potassium iodide is so soluble in water and water turns the red to blue again, it seems more probable that the amount of potassium iodide is increased in the red starch iodide and dissolved out again by the water to reform the blue; that is, it requires considerable concentration of the potassium iodide solution to keep the requisite amount of potassium iodide in the starch red. As Mylius gives the grouping (I.) to starch blue, so I would suggest the grouping (II.) for starch red; the former grouping answers to the ratio 1 : 4, the latter 1 : 2.



I.



II.

It may be, too, that the amount of iodine also changes.

It is a very difficult problem to isolate the starch red, but the following experiments tend to corroborate the above theory :

If starch iodide blue is washed with a concentrated solution of potassium iodide the blue changes to red. This action is hastened and the color deepened by washing with 95 per cent alcohol, or better with absolute alcohol. The alcohol seems to help by removing water, for dilute alcohol restores the blue color as well as water. As a preliminary experiment, about 0.25 gram of starch in 1 per cent solution was treated with 1 cc. of N/10 iodine, and the resulting starch iodide precipitated by dilute sulphuric acid, washed with water, and then washed with a concentrated solution of potassium iodide and with absolute alcohol. It was washed ten to twenty times with the alcohol, but even then free iodine and potassium iodide continued to appear in the alcohol. This red

precipitate was washed into a beaker, arsenite added, and the analysis made as with the starch iodide blue, the starch being precipitated by alcohol.

	I. N/100 iodine. cc.
50 cc. N/100 arsenite	50.00
Excess of arsenite solution	43.60
Iodine of starch red	6.40
125 cc. N/100 arsenite	125.00
Excess of arsenite	7.80
Total iodine freed by iodic acid	117.20
$\frac{5}{8} \times 117.20 =$ iodine of potassium iodide freed by iodic acid	97.67
50 cc. N/100 arsenite	50.00
Iodine of potassium iodide in iodine solu- tion introduced and in starch red	47.67
Iodine of potassium iodide in iodine solu- tion introduced	45.08
Iodine of potassium iodide in starch red	2.59

The ratio of potassium iodide to iodine is $2.59 : 6.40 = 1 : 2.47$.

The presence of free iodine in the alcohol washings appears to be explained by the supposition that the alcohol washed it out from the starch iodide blue or red in the presence of the increased amount of potassium iodide. This view is supported by the fact that in the analysis almost half of the iodine originally present in the starch blue had been washed out, being reduced from about 10 cc. of N/100 iodine to 6.4 cc.

Three more analyses were now made with larger quantities. Into 100 cc. of a 0.5 per cent solution of starch 20 cc. of N/100 iodine were run and the starch blue precipitated by sulphuric acid and filtered. The precipitate was washed with concentrated potassium iodide and with 95 per cent alcohol. The washing with alcohol was continued until the precipitate began to turn purplish and the analysis carried out as before.

	cc. N/100 iodine.		
	II.	III.	IV.
50 cc. N/100 arsenite	50.00	50.00	50.00
Excess of arsenite solution	32.35	31.73	37.05
Iodine of starch red	17.65	18.27	12.95
115 cc. N/100 arsenite	115.00	115.00	115.00
Excess of arsenite	5.95	6.38	1.40
Total iodine freed by iodic acid	109.05	108.62	113.60
$\frac{2}{3} \times 109.05, 108.62, \text{ and } 113.60 = \text{iodine}$ of potassium iodide freed by iodic acid	90.88	90.52	94.67
50 cc. N/100 arsenite	50.00	50.00	50.00
Iodine of potassium iodide in iodine solu- tion introduced and in starch red	40.88	40.52	44.67
Iodine of potassium iodide in iodine solu- tion introduced.	33.37	32.73	38.22
Iodine of potassium iodide in starch iodide red	7.51	7.79	6.45
Ratio of potassium iodide to iodine	7.51 : 17.65 : 1 : 2.35	7.79 : 18.27 : 1 : 2.35	6.45 : 12.95 : 1 : 2.01

When the difficulties of the analysis are considered, including the fact that the starch red had to be washed until it had commenced to change back to the blue compound so as to be sure that no potassium iodide was held mechanically, the results of the analyses may be taken as corroborative of the theory. My theory of the structure of the starch iodide red as compared with the starch blue is, then, that in the presence of a sufficiently concentrated solution of potassium iodide, helped also by the absence of water, the group $(KI.I_4)$ changes to two groups of $(KI.I_2)$, and that the addition of water removes 1 molecule of potassium iodide, reducing the two latter groups to the former, thus explaining the change of color to red or blue according as potassium iodide is introduced in excess or washed out with water.

In a previous article¹ it has been shown that amidulin is intermediate between starch and dextrin. This fact is also

¹ Am. J. Sci., 1902, p. 379.

shown by the relation of amidulin to potassium iodide, for amidulin iodide blue is far more susceptible to its influence than is starch iodide, that is, solutions of potassium iodide of much less concentration give a reddish tinge to amidulin blue. Concentrated potassium iodide gives to erythrodextrin red an orange-brown tone, not so yellowish, but more reddish, and far deeper than the color of iodine in potassium iodide in blank.

Some further interesting results come out of these analyses. Of the 0.5 per cent starch paste employed it took 200 cc. to completely precipitate 40 cc of N/100 iodine solution. If 50 cc. of N/100 iodine were added a distinct yellow color was visible in the filtrate, or supernatant liquid. The one analysis mentioned showed that all the starch came down with the starch iodide. Mylius¹ found a ratio of iodine to starch iodide which answered to the formula $[(C_6H_{10}O_5)_4I]_4.HI$. Rouvier² found a ratio in his starch iodide leading to $(C_6H_{10}O_5)_8I$. He precipitated with an excess of starch and thought that the excess of starch explained the difference, as Mylius precipitated with iodine in excess. In my experiments the starch was in excess, and taking the fact into account that 1 gram of the starch paste used was a bare excess for 40 cc. of N/100 iodine, the starch iodide answers to $[(C_6H_{10}O_5)_{16}I]_4.HI$, that is, my starch paste, which was rather viscous, appears to have been twice as large a molecular body as Rouvier's starch and four times as large as Mylius' starch.

Wholly apart from the consideration of theory, the influence of iodides upon the delicacy of the starch blue test for iodine is sometimes a matter of considerable practical importance analytically. In standardizing certain thiosulphate solutions by iodine in presence of acid, Norris and Fay,³ finding that they obtained a higher standard at the temperature of ice-water than at the ordinary laboratory temperature, have attributed that fact to the greater delicacy of the starch blue reaction at low temperatures. It is evident that these investigators have mistaken the occasion of variation in their

¹ Ber. d. chem. Ges., 20, 688.

² Compt. rend., 114, 1366-1367.

³ This JOURNAL, 23, 125.

standards in overlooking the well-known influence of potassium iodide, always present in standard iodine solutions, upon the starch iodide reaction. Under the actual conditions of standardizing, the use of even 10 cc of N/10 iodine as ordinarily made up in potassium iodide implies the simultaneous introduction of, or production of, over 0.3 gram of potassium iodide. Experience shows that the presence of this amount of potassium iodide in so much as 300 cc. of total dilution is sufficient to make the readings by the starch indicator quite as delicate at ordinary temperatures as at the temperature of ice-water, and the addition of an excess of potassium iodide above this amount does not render the reading any sharper. The following table demonstrates the exact agreement of the readings under the two sets of conditions, the presence of sufficient potassium iodide at ordinary temperature, and the presence of 10 cc. of hydrochloric acid at the temperature of ice-water :

Table I.

Volume. cc.	Potassium iodide. (0.1-0.3 gram.)	Hydrochloric acid. (Sp. gr. 1.12.) cc.	Temperature.	Pure potato starch solution. cc.	Sulphuric acid. cc.	N/10 iodine. cc.	Thiosulphate. cc.
300		10	6 ⁰¹	2	•	25	35.26 ²
"		10	6 ⁰	2	•	25	"
"	1 crystal	..	23 ⁰	2	5	25	"
325	1 "	..	23 ⁰	2	5	25	"

The sharpness of the readings in the two first titrations is not due solely to the low temperature or to the presence of the hydrochloric acid, but mainly to the potassium iodide introduced and formed, which fact is clearly shown in the following table, which gives the results obtained when a N/1000 solution of iodine was used under varying conditions :

¹ The temperature was taken at the end of the titration.

² The thiosulphate was run in 0.1-0.2 cc. at a time until near the end, and then by drops.

Table II.

Volume. cc.	Potassium iodide not exceeding 0.3 gram.	Hydrochloric acid cc. (Sp. gr. 1.12).	Pure potato starch solution. cc.	Temperature.	N/1000 iodine. cc.	Color.
100	1 crystal	..	2	A 23°	0.45	Faint-blue
200	"	..	2	23°	0.65	"
300	"	..	2	23°	0.80	"
400	"	..	2	23°	1.30	"
300	"	..	2	B 23°	0.80	"
300	"	..	2	5 ⁰¹	0.70	"
300		..	2	C 23°	9.20	"
300		..	2	5 ⁰¹	1.50	"
300		I	2	D 23°	4.15	"
300		10	2	23°	4.00	"

In the absence of a sufficient amount of potassium iodide (C) the influence of temperature is very noticeable, but in the presence of potassium iodide (B), though not exceeding 0.3 gram, and at a dilution of 300 cc., no closer reading could be obtained at the temperature of ice-water than at the ordinary laboratory temperature. In the absence of potassium iodide, even at the temperature of ice-water, the reading was almost twice as large as that obtained in the presence of potassium iodide at the ordinary temperature. An increase in the amount of hydrochloric acid did not materially increase the sharpness of reading (D), and the reading was fully five times as large as that obtained by the influence of potassium iodide. The first four experiments (A) show that the total volume has considerable proportionate influence upon the amount of iodine needed for a reading. These experiments are in agreement with Lonnes¹⁹ work.

¹ At the temperature of ice-water the blue color had a greenish tinge.

² Ztschr. anal. Chem., 1894, p. 409.

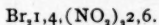
Contributions from the Chemical Laboratory of Harvard College.

ON THE DIBROMDINITROBENZOLS DERIVED FROM PARADIBROMBENZOL.

[SECOND PAPER.¹]

BY C. LORING JACKSON AND D. F. CALHANE.

The mixture of isomeric dibromdinitrobenzols obtained by the action of nitric acid and sulphuric acid on paradibrombenzol was first studied by Austen,² who isolated two of the isomers, which he distinguished by the prefixes α and β . Later he established the constitution³ of the β -substance as



The structure of Austen's α -isomer has been determined recently by one of us and Wheeler⁴ as $\text{Br}_2\text{I},4,(\text{NO}_2)_2,3$.

In his first paper on this subject Austen mentioned a body melting near 120° , which he took to be the third isomer, but in the last of his papers in the *Berichte* he announced his conclusion that this substance was a mixture and not a new isomeric form. In continuing the work we have succeeded in obtaining this third isomer, and, as it melts at 127° , it is not improbable that it formed part, at least, of the body observed by Austen. We obtained it from the mother-liquors left after the α -isomer had crystallized from the solution of the crude product in glacial acetic acid. For this purpose the solid matter was precipitated with water, and, after being dried, extracted with ice-cold ether, in which the new γ -isomer was practically insoluble, whereas the β -modification went into solution. The yield was less than 10 per cent of the crude material, the yield of the α -isomer somewhat over 5 per cent, and although it is obvious that considerable amounts of these isomers remained in the mother-liquors, so that these yields are far from representing the quantities formed, yet there is no doubt that the β -isomer is the principal product of the reaction.

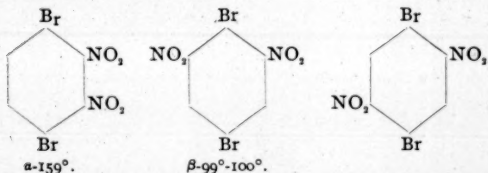
¹ First paper, Calhane and Wheeler: *This JOURNAL*, 22, 451.

² *Ber. d. chem. Ges.*, 8, 1183; 9, 621, 918.

³ *Am. J. Sci.*, [3], 16, 46.

⁴ Calhane and Wheeler: *Loc. cit.*

There are only three dinitro compounds which can be derived from paradibrombenzol,



and, as the constitution of the α - and of the β -isomers has been determined, it follows that the new isomer must have the structure $\text{Br}_{1,4}(\text{NO}_2)_{2,5}$. We thought it worth while, however, to test this inference by experiment, and accordingly reduced the new dibromdinitrobenzol to a diamine with tin and hydrochloric acid, and then oxidized this diamine with a mixture of sulphuric acid and potassic dichromate, when we obtained the dibromquinone $\text{C}_6\text{H}_2\text{O}_2\text{Br}_2$, melting at 187° to 188° , made by Sarauw¹ from dibromhydroquinone by oxidation. As our sample of the quinone was made from paradibrombenzol, its constitution must be $\text{Br}_{1,4}\text{O}_{2,5}$, and the constitution ascribed to our isomer, $\text{Br}_{1,4}(\text{NO}_2)_{2,5}$, is confirmed. We may add that the diamine gave, with ferric chloride and sulphuretted hydrogen in acid solution, the blue color characteristic of paradiamines.

The 1,4-dibrom-2,5-dinitrobenzol melts at 127° , so that its melting-point lies between those of the vicinal (α) form, 159° , and the unsymmetrical (β) form, 99° - 100° . It is a decidedly reactive substance, and we have studied some of its derivatives, but the large outlay of time necessary to prepare it in quantity has prevented us from carrying this work so far as we could have wished.

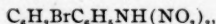
The 1,4-dibrom-2,5-phenylenediamine obtained by reducing the dinitro compound with tin and hydrochloric acid (zinc dust and acetic acid give a less pure product) melts at 183° to 184° and forms a chloride, $\text{C}_6\text{H}_4\text{Br}_2(\text{NH}_2\text{Cl})_2$.

Alcoholic ammonia at 100° gives with the γ -dibromdinitro-

¹ Ann. Chem. (Liebig), 209, 112.

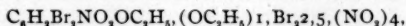
benzol the 1,4-dibrom-2-nitro 5-aminobenzol (2,5-dibrom-4-nitraniline) $C_6H_2Br_2NO_2NH_2$, melting at 174° to 175° , by the replacement of one nitro by an amino group, but the yield is very small. Ammonic bromide is also formed in the reaction, which points to another organic product. This, however, we did not succeed in isolating from the viscous residues.

Aniline gives the 1-brom-4-anilido-2,5-dinitrobenzol,



an orange-red compound melting at 153° to 154° . We did not succeed in finding a second modification of this substance, although we looked carefully for it, because the occurrence of two modifications of such nitranilido compounds has been observed¹ several times in this laboratory.²

Sodic ethylate in the cold replaces one of the nitro groups by an ethoxy group forming the dibrominitrophenetol,



which melts at 126° . It is curious that this substance melts only 1° below the dibromdinitrobenzol from which it was formed, and resembles it so closely in crystalline form and solubility that the only indication of a reaction was the formation of sodic nitrite. The reaction runs quickly and neatly. No sodic bromide could be detected among the products. If the sodic ethylate acted at the boiling-point of alcohol, a bromine atom was also replaced by an ethoxy group, giving a bromnitrodiethoxybenzol, $C_6H_3BrNO_2(OC_2H_5)_2$, melting at 103° to 104° . This compound may be either a resorcin ether, $(C_2H_5O)_2, 1, 3, Br_4, NO_2, 6$, or a pyrocatechin ether, $(C_2H_5O)_2, 1, 2, Br_4, NO_2, 5$. An attempt to determine its constitution with an insufficient amount of substance seemed to show that it was a pyrocatechin ether, because an alcoholic solution of the free phenol gave a precipitate with an aqueous solution of plumbic acetate. We do not, however, attach much weight to this

¹ Jackson and Bentley: *This JOURNAL*, 14, 348; Jackson and Herman: *Ibid.*, 16, 35; Jackson and Cohoe: *Ibid.*, 26, 5.

² The observation, that bromine was replaced when aniline acted with the γ -isomer, whereas the nitro group was replaced when ammonia was used, has no real significance, because probably with both reagents parallel actions affecting the bromine and the nitro group took place, but in each case we failed to isolate more than one of the products.

experiment, as, when performed on so small a scale, it was impossible to guard with certainty against the numerous sources of error. On the other hand, the atom of bromine replaced to form a pyrocatechin ether is in the meta position to the nitro group, whereas the atom of bromine replaced, if a resorcin ether was formed, is ortho to the nitro group; as all previous experience in such replacements has shown that the loosening effect of a nitro group in the meta position is much slighter than that of one in the ortho position, we think it much more probable that the substance is a resorcin ether than a pyrocatechin derivative in spite of the experimental evidence; accordingly we have assigned this constitution to it provisionally.

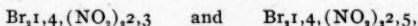
An experiment was tried on a small scale with a boiling solution of sodic hydrate and the dibromdinitrobenzol, which showed that both sodic nitrite and bromide were formed, and that, therefore, the reaction runs in essentially the same way as that with hot sodic ethylate. Lack of material prevented us from studying the organic products, which promised to be of only slight interest. For the same reason the product of the action of sodic malonic ester on the dibromdinitrobenzol was not studied, although a preliminary experiment showed that a red salt was formed.

We have also studied the behavior of the α -1,4-dibrom-2,3-dinitrobenzol with sodic ethylate in the cold. The reaction ran much less rapidly and neatly than that with the 2,5-dinitro compound, sodic nitrite was formed with little more than a trace of sodic bromide, and the product consisted principally of unmanageable tars. We succeeded, however, in isolating a dibromnitrophenetol, $C_6H_4Br_2NO_2 \cdot OC_2H_5$, formed by the replacement of one nitro group by an ethoxy group, which must have the constitution $Br1,4,NO_2,2,(OC_2H_5)_3$. It melts at 45° and the yield was 18 per cent. In spite of many experiments we are unable to give any account of the other products of the reaction. If the reaction between the 1,4-dibrom-2,3-dinitrobenzol and sodic ethylate was carried on at the boiling-point of alcohol, nearly 15 per cent of the total amount of bromine was converted into sodic bromide, so that

this reaction is undoubtedly similar to the one observed with the γ -compound under the same conditions. With alcoholic ammonia at 100° about 13 per cent of the bromine in the α -body was converted into ammonic bromide. This result corrects the statement of Austen¹ that no ammonic bromide was formed under these conditions.

The 2,3-dinitro compound, when boiled with an aqueous solution of sodic hydrate, gave, by the replacement of one nitro group by hydroxyl, the 2,5-dibrom-6-nitrophenol, which melts at 77° and forms the yellow, slightly soluble barium salt, $(C_6H_3BrNO_2O)_2Ba \cdot 3H_2O$. Only a very small amount of sodic bromide was formed in this process.

In their behavior with reagents the two isomers,



show a strong similarity. In both cases a nitro group is more easily replaced than the bromine, as shown by the action of a cold solution of sodic ethylate, but one of the atoms of bromine can be replaced without much difficulty. It was observed repeatedly, however, that the 2,3-dinitro body was much more sluggish in its action than the 2,5-dinitro compound. The only difference in the structures of these isomers is that in the first the nitro group which is replaced by another radical, is in the ortho, in the second in the para position to the other nitro group, and these observations, therefore, are in harmony with the quantitative results of C. A. Lobry de Bruyn and Alph. Steger,² who found that the speed of substitution of a nitro group by the ethoxyl radical is nine times as great in paradinitrobenzol as in orthodinitrobenzol.

In our work with both these dibromdinitrobenzols we observed no sign of the replacement of bromine by hydrogen in reactions with sodic ethylate, the search for which had led us to undertake the study of these compounds. Tin and hydrochloric acid also failed to remove the bromine from the γ -2,5-dinitro compound, but this is not surprising, as we can

¹ Ber. d. chem. Ges., 9, 632.

² Rec. trav. chim. Pays-Bas, 18, 9; Steger: *Ibid.*, 13.

find¹ no case of such a removal of bromine from paradinitro compounds.

EXPERIMENTAL PART.

γ-Isomer.

Isolation of 1,4-Dibrom-2,5-dinitrobenzol.—The crude product from which this substance was extracted was made by the action of fuming nitric acid and strong sulphuric acid upon paradibrombenzol, as follows:² Two hundred grams of paradibrombenzol (made by the method of Jannasch³) were dissolved in 650 grams of fuming nitric acid of sp. gr. 1.505, and mixed with the same volume of strong sulphuric acid, which caused the precipitation of a red oil. Disregarding this, the mixture was warmed, and as soon as a violent action set in the source of heat was removed until it had come to an end. The liquid was then boiled gently, for three hours, in a flask closed with a porcelain crucible, after which it was allowed to cool, and, before the oil had solidified, poured into a large quantity of water contained in an evaporating dish for convenience in removing the solid, which formed after standing over night. The crude product amounted to 246 grams.

This product of the reaction, after washing with water, was dissolved in glacial acetic acid (85 cc. of acid to each 50 grams of the substance), and allowed to stand in a cold place, when a quantity of the α -isomer ($\text{Br}_2, 1,4, (\text{NO}_2)_2, 3$) crystallized out. The mother-liquor from this, when precipitated with water, furnished a mixture of all three isomers, which was treated as follows: Fifty grams of this mixture were digested with 175 cc. of ether for one hour, the mixture being cooled with snow or ice during this time, and stirred continually, taking care to break up the lumps as much as possible to aid the solvent action of the ether. The undissolved portion was filtered out, and in this way 38 grams, insoluble in ether, were obtained from 300 grams of the crude material. The ethereal solution was evaporated and the residue put aside to be worked up for

¹ This JOURNAL, 18, 465.

² Austen: Ber. d. chem. Ges., 9, 621; Calhane and Wheeler: This JOURNAL, 22, 451.

³ Ber. d. chem. Ges., 10, 1355.

the β -isomer in the future. The 38 grams of impure dibromdinitrobenzol, insoluble in ether, melted at 105° . They were divided into two lots of 19 grams, and each of these recrystallized from 250 cc. of alcohol, which gave 22 grams of nearly pure product from the 38 grams of crude substance. One more crystallization from alcohol raised the melting-point to 127° , where it remained constant. It was dried at 100° and analyzed with the following result:

I. 0.2503 gram substance gave, by the method of Carius, 0.2892 gram of argentic bromide.

II. 0.2656 gram substance gave 22.2 cc. N at 31° and 761 mm.

	Calculated for $C_6H_2Br_2(NO_2)_2$	I.	Found.	II.
Br	49.08	49.16		
N	8.59	...	...	9.03

The substance is, therefore, the third dibromdinitrobenzol derived from paradibrombenzol, and has the constitution $Br_2,1,4,(NO_2)_2,5$, as proved later in this paper.

Properties of the 1,4-Dibrom-2,5-dinitrobenzol.—When crystallized from a mixture of benzol and alcohol it forms at first short prisms with two edges truncated by a basal plane and terminated by two planes. As the crystals grow, one of the terminal planes becomes developed at the expense of the other, so that the final form consists of long, rather slender, prisms, terminated by a single plane at an oblique angle. The crystals apparently belong to the monoclinic system, and show a tendency to twin by uniting along the edges. It was also observed in arborescent forms having a general resemblance to those of ammoniac chloride. It is white with a pale-yellowish tinge, and melts at 127° . It is soluble in benzol, chloroform, acetone, or carbonic disulphide; slightly soluble in ether; slightly soluble in cold, soluble in hot alcohol; slightly soluble in cold, readily soluble in hot amyl alcohol, or glacial acetic acid; practically insoluble in cold, sparingly soluble in hot ligroin; practically insoluble in water. Strong hydrochloric acid or strong sulphuric acid has no apparent action, hot or cold. Fuming nitric acid does not seem to af-

fect it when cold, but dissolves it when hot. The action of sodic hydrate upon it is described later in this paper. The best solvent for it is alcohol.

Reduction of the 1,4-Dibrom-2,5-dinitrobenzol.

The reduction was made both with tin and hydrochloric acid, and with zinc dust and acetic acid. The first method is to be preferred, as it gives a white, pure product at once, whereas the product from the action of zinc and acetic acid is usually highly colored, and can be purified only with difficulty.

Three grams of the dibromdinitrobenzol were mixed in a flask with 150 cc. of strong hydrochloric acid and about 30 cc. of alcohol. An excess of granulated tin and a piece of platinum foil were then added and the mixture heated on the steam-bath under a return condenser until all the organic matter had gone into solution, which usually occurred in eight hours. At the beginning of the process the crystals took on a deeper yellow color, and later a white, flaky body was precipitated, which afterward went into solution. After the reduction had come to an end the clear solution was poured off from the excess of tin and, on cooling, began to deposit white, feathery crystals of a double salt of tin, which were dissolved by the addition of more water, and then sodic hydrate was added until the liquid showed a strong alkaline reaction. The quantity of sodic hydrate used was not enough to redissolve all the hydrates of tin, as this would have given an inconveniently large volume of liquid. The alkaline liquid with the suspended hydrate of tin was next extracted with ether, the ether extract shaken with aqueous sodic hydrate, which dissolved out the hydrate of tin taken up by the ether, and the clear ethereal solution allowed to evaporate. The residue was a white, waxy substance, which turned brown on exposure to the air. As it still contained a quantity of inorganic matter it was treated with hot benzol and filtered; the deep-yellow filtrate, on cooling, deposited pearly plates, which showed the melting-point 183° to 184° , and as repeated crystallization from benzol did not raise this melting-point, the substance was obtained pure without recrystallization.

The reduction with zinc and acetic acid was carried on as follows: Three grams of the 1,4-dibrom-2,5-dinitrobenzol mixed with 60 cc. of glacial acetic acid somewhat diluted with water were warmed gently on the steam-bath, and to the mixture zinc dust was added from time to time until 9 grams had been used and the heating had continued for four hours. During this time the color of the solution changed from yellow to deep-purple. Upon the addition of a large quantity of water to the product a black precipitate was formed, which weighed about 2 grams, and, therefore, contained the larger part of the reduction-product. The filtrate had a dark-purple color, but yielded very little organic matter, when extracted with ether direct, or when the ether extraction was preceded by treatment with sodic hydrate. The black precipitate was dissolved in boiling benzol and filtered hot. The filtrate, which had a deep mahogany-brown color, deposited long, brown needles as it cooled, which retained their color even after several crystallizations from benzol with the aid of bone-black. This strong and persistent color, and the fact that the substance crystallized in needles instead of the plates observed with the diamine prepared by tin and hydrochloric acid, made us suspect at first that this substance was not the diamine, but on determining its melting-point we found it to be 181° to 182° , and this was so near that of the diamine (183°) that it seemed more probable that it was really the diamine and that the color and difference in crystalline appearance were due to the presence of a clinging impurity. This latter view was proved to be correct by substituting chloroform for benzol in the purification of the product, when, even at the first crystallization, the color was removed in great measure, and it crystallized in plates instead of needles. A second crystallization from chloroform gave white plates melting at 183° , identical with those prepared by tin and hydrochloric acid. In describing the preparation of the diamine by zinc and acetic acid we mentioned that the product was treated with hot benzol to dissolve the brown diamine; there was left after this treatment a bluish-purple mass insoluble in benzol, which was amorphous and bulky. We are inclined to

think that this consisted principally of the diamine protected from solution in the benzol by a still larger amount of the impurity contained in the brown needles, since this bluish-purple substance also melted at about 183° . It may be added that the brown crystals were decolorized by reduction with tin and hydrochloric acid, yielding the diamine, crystallizing in plates, and melting at 183° .

A sample of the diamine dried at 100° gave the following result on analysis :

0.1395 gram substance gave, by the Carius method, 0.1971 gram argentic bromide.

	Calculated for $C_6H_2Br_2(NH_2)_2$	Found.
Br	60.15	60.12

This analysis is confirmed by the analysis of the chloride of the diamine given later.

Properties of the 1,4-Dibrom-2,5-phenylenediamine.—When crystallized from chloroform it appears in large, white plates¹ with a pearly luster, which turn brown on exposure to the light. A freshly prepared and recrystallized sample melted at 183° to 184° . When it melts the substance is converted into a dark-blue body, possibly identical with the impurity which gave so much trouble in purifying the product from zinc and acetic acid. We have not attempted to investigate this compound on account of the difficulty of preparing the diamine on a large scale. The diamine is soluble in ether or acetone ; slightly soluble in alcohol, benzol, chloroform, glacial acetic acid, or ligroin, when cold, more soluble in each of these solvents when hot. Chloroform is the best solvent for it, but in bringing it into solution long or vigorous heating should be avoided, as this causes decomposition. Strong hydrochloric acid has no action on it, when cold. When the mixture is heated, a small amount of the diamine is dissolved, but the action is too incomplete to use this method to advantage in forming salts. Strong nitric acid acts vigorously, forming a yellow body with a marked quinone odor. Fuming nitric acid acts with explosive violence, converting the diamine into a

¹ As mentioned above, when impure it may crystallize in needles instead of plates.

black tar. Strong sulphuric acid has no effect in the cold, but dissolves the diamine when hot. On cooling, a crystalline body separates, which is perhaps the sulphate. With sodic hydrate there is no apparent action, whether hot or cold. Two experiments with ferric chloride showed that it acted on the diamine dissolved in acetone, but no definite oxidation-products could be isolated with the small amount of material at our disposal. The diamine, when mixed with ferric chloride and sulphuretted hydrogen in dilute acid solution, gave a purple product similar to Lauth's violet.

Chloride of the 1,4-Dibrom-2,5-Phenylenediamine, $C_6H_2Br_2(NH_2Cl)_2$.—This salt was prepared by saturating with hydrochloric acid gas a solution of the diamine in dry benzol. The white precipitate thus obtained was washed with benzol, dried in a desiccator, and analyzed with the following result: 0.2009 gram substance gave, by the Carius method, 0.3927 gram of the mixture of argentic chloride and argentic bromide.

	Calculated for $C_6H_2Br_2(NH_2Cl)_2$	Found.
Cl and Br	68.14	68.14

Properties.—The chloride of the diamine, when crystallized from a boiling, moderately dilute solution of hydrochloric acid, forms long, slender, white needles with a vitreous luster. It is not very stable, as a specimen of it turned green in a single night when exposed to the air of the laboratory. Upon heating it seems to be decomposed into hydrochloric acid and the free diamine, which sublimes. It dissolves in boiling water apparently without decomposition, but is only very slightly soluble in boiling strong hydrochloric acid. The best solvent for it is somewhat diluted hydrochloric acid.

Constitution of the Third Isomeric Dibromdinitrobenzol Derived from Paradibrombenzol.

Although the constitution of this isomer was essentially established by exclusion¹ as $Br_{2,1,4},(NO_2)_{2,5}$, we thought it necessary to confirm this inference by experiment. Accord-

¹ See the introduction to this paper.

ingly the diamine, made by the reduction of the dibromdinitrobenzol with tin and hydrochloric acid, was oxidized as follows: Eight grams of strong sulphuric acid were diluted with 32 cc. of water and the mixture cooled to zero by immersing it in ice. One gram of the dibromphenylenediamine was dissolved in this and then 1 gram of powdered potassic dichromate was added in small portions at a time with careful stirring. The mixture was allowed to stand over night, after which it was cooled again to zero, and 1 gram more of potassic dichromate added with the same precautions as before. It was now allowed to stand three or four hours and then extracted with ether. Upon allowing the ether to evaporate, yellowish-brown grains were obtained with an unmistakable quinone smell, which, after recrystallization from alcohol, were converted into short, golden-yellow prisms melting at 187° to 188° . The substance, therefore, is evidently the dibromquinone, which melts at 188° , obtained by Sarauw¹ by oxidizing dibromhydroquinone with ferric chloride, and this inference is confirmed by the agreement in the other properties of the two bodies, both of which show similar solubilities and sublime in small needles. As Sarauw prepared this substance from hydroquinone, and we made it from para-dibrombenzol, it can have only the structure $\text{Br}_{1,4}\text{O}_{2,5}$, and it follows, therefore, that the constitution of our dibromdinitrobenzol must be $\text{Br}_{1,4}(\text{NO}_2)_{2,5}$. This constitution was also confirmed by the fact that our diamine, when treated with sulphuretted hydrogen and ferric chloride in acid solution, gave the deep-blue coloration characteristic of the para-diamines.

Action of Alcoholic Ammonia on 1,4-Dibrom-2,5-dinitrobenzol.

Our work on this subject is not so complete as we could wish, since the experiments here described exhausted our supply of the γ -isomer, and the importance of this part of the research was not sufficient to justify the large expenditure of time necessary to provide a new supply.

Half a gram of the dibromdinitrobenzol was sealed in a tube with 15 to 20 cc. of a saturated solution of ammonia in abso-

¹ Ann. Chem. (Liebig), 209, 112.

lute alcohol and heated to 100° for five or six hours. At the end of this time the tube was opened and its contents, after driving off the alcohol, washed thoroughly with water. The wash-water gave a strong test for a bromide with argentic nitrate and nitric acid. Upon extracting the washed residue with ligroin a substance melting between 135° and 139° was obtained, but in too small amount for investigation. The portion insoluble in ligroin was next treated with benzol containing a little alcohol, and in this way another compound was extracted, which, after recrystallization, melted constantly at 174° to 175°. It was dried at 100° and analyzed with the following result:

0.1480 gram substance gave, by the Carius method, 0.1856 gram argentic bromide.

	Calculated for $C_6H_3Br_2NO_2NH_2$	Found.
Br	54.05	53.37

The substance was formed, therefore, by the replacement of one nitro by an amino group, and is 1,4-dibrom-2-nitro-5-aminobenzol (2,5-dibrom-4-nitraniline). The yield was very small, so that the principal product was contained in the residue insoluble in benzol, and was probably formed by the reaction which produced the ammonic bromide found in the wash-water.

The 1,4-dibrom-2-nitro-5-aminobenzol crystallizes in deep brownish-yellow prisms, which melt at 174° to 175°. It is sparingly soluble in cold, more soluble in hot benzol. The benzol solution, if treated with hydrochloric acid gas, gives a precipitate of a salt in yellow needles.

*Action of Aniline on the 1,4-Dibrom-2,5-dinitrobenzol. Brom-anilidodinitrobenzol, $C_6H_4BrC_6H_4NH(NO_2)_2$. BrI,
 $C_6H_5NH_4(NO_2)_2$, 2,5.*

In spite of the fact that the dinitro compound dissolved in aniline even in the cold, imparting a deep-red color to the liquid, some preliminary experiments had indicated that the action took place slowly even at 100°. We proceeded, therefore, as follows: The substance was heated on

the steam-bath with an excess of aniline for three or more hours, and the product was allowed to stand over night. The excess of aniline was then removed by washing with acid and water, and on testing the wash-waters it was found that bromide of aniline had been formed. The residue, insoluble in water, was a red powder, which was purified by crystallization from alcohol containing a little benzol, until it showed the constant melting-point 153° to 154° , when it was dried at 100° and analyzed with the following result :

0.2463 gram substance gave, by the Carius method, 0.1365 gram argentic bromide.

	Calculated for $C_6H_2Br(C_6H_5NH)(NO_2)_2$	Found.
Br	23.66	23.58

Properties of Bromanilidodinitrobenzol,

$BrI, C_6H_5NH_4, (NO_2)_2, 5$.--It crystallizes from alcohol and benzol in six-sided plates not far removed in shape from regular hexagons, which later develop into prisms belonging to the monoclinic or orthorhombic system. Its color is orange-red, somewhat redder than that of potassic dichromate. We could find no evidence that it existed in more than one modification, although we tried in many ways to convert it into a second form, since Cohoe and one of us¹ have found that the bromanilidodinitrobenzol, $BrI, C_6H_5NH_3, (NO_2)_2, 4, 6$, occurs in a red and a yellow modification. It melts at 153° to 154° . It is soluble in ether, benzol, chloroform, glacial acetic acid, or carbonic disulphide; sparingly soluble in methyl or ethyl alcohol, whether cold or hot; very sparingly soluble in ligroin. The best solvent for it is ethyl alcohol containing a little benzol. Strong sulphuric acid dissolves it slowly with a deep-violet color, which changes to brown on heating. Nitric acid in the cold has no perceptible effect on it. When hot it dissolves it slowly, forming a deep-orange solution from which nothing is precipitated by dilution. Fuming nitric acid acts vigorously on it in the cold, forming a brownish-black solution, from which water throws down a yellowish-green precipitate. Sodic hydrate has little or no action on it even in hot solution.

¹ This JOURNAL, 26, 1.

An attempt to prepare a dianilido body by the action of aniline at higher temperatures (175° to 180°) gave an unpromising-looking product, and, as the substance would be of but little importance, we did not pursue this part of the subject further.

Action of a Cold Solution of Sodic Ethylate on the 1,4-Dibrom-2,5-dinitrobenzol.

Five grams of the dibromdinitrobenzol were dissolved in benzol and mixed with the solution of sodic ethylate prepared by treating 0.7 gram of sodium with absolute alcohol, that is, 2 molecules of sodic ethylate were used for each molecule of the dibromdinitrobenzol. As soon as the ethylate was added a flocculent precipitate was formed, and the solution took on a deep wine-red color, which increased in intensity on standing. To make sure that the reaction was complete the mixture was kept at ordinary temperatures in a corked flask for over thirty-six hours, at the end of which time a brick-red precipitate had collected in the bottom of the flask. This was filtered out and proved to be entirely soluble in water. It gave no test for sodic bromide and contained not more than a trace of organic matter, but with an acidified solution of potassic iodide and starch paste it gave a strong test for a nitrite, and upon adding dilute sulphuric acid a copious evolution of red, nitrous fumes was obtained. It was evident, therefore, that the precipitate was principally sodic nitrite. The alcohol and benzol filtrate from this precipitate of nitrite was allowed to evaporate spontaneously and the brownish-white residue washed with water, after which it was recrystallized from hot alcohol until it showed the constant melting-point 126° . The melting-point was, therefore, almost identical with that of the original substance, 127° , and the solubility in alcohol and its appearance were so similar to those of the dibromdinitrobenzol that the formation of the sodic nitrite was the only indication that a reaction had taken place. It was dried at 100° and analyzed with the following result :

0.3082 gram substance gave 12 cc. N at 20° and 760 mm. pressure.

	Calculated for $C_6H_2Br_2NO_2OC_2H_5$	Found.
N	4.31	4.45

The substance contains bromine. It is evidently formed by the replacement of one of the nitro groups by an ethoxy group. The yield was somewhat over 4 grams from the 5 grams of dibromdinitrobenzol, that is, more than 80 per cent of that required by theory.

Properties of Dibromnitrophenetol, $C_6H_2Br_2NO_2OC_2H_5$. $(C_2H_5O)_1Br_{2,5}NO_{2,4}$.—When crystallized from hot alcohol it forms at first groups of radiating white needles, which in time develop into slender prisms with blunt ends. It melts at 126°. It is freely soluble in benzol, toluol, chloroform, acetone, or carbonic disulphide; somewhat less soluble in ether or glacial acetic acid; very slightly soluble in ethyl or methyl alcohol when cold, soluble in them when hot; almost insoluble in cold ligroin or amyl alcohol, soluble in these liquids when hot. The best solvent for it is alcohol. Strong hydrochloric acid has no visible effect on it, even when hot. Strong nitric acid also seems to have no action on it in the cold, but when hot dissolves it slowly with a yellow color. On cooling, crystals are deposited which look like those of the original body. Fuming nitric acid dissolves the substance at once, even in the cold. On diluting this solution, a white precipitate is formed. Strong, cold sulphuric acid turns the substance deep golden-yellow with partial solution. When hot the acid dissolves it slowly. Sodic hydrate has no apparent effect, even when hot. Cold aniline dissolves it easily, but on removing the aniline with dilute hydrochloric acid the residue is white, so that the aniline has probably acted merely as a solvent.

Action of a Hot Solution of Sodic Ethylate on the 1,4-Dibrom-2,5-dinitrobenzol.

In an experiment similar to that described in the preceding section, except that the mixture of sodic ethylate and

dibromdinitrobenzol was heated for two hours after standing in the cold, we obtained in addition to the substituted phenetol melting at 126° another substance melting at 103° to 104° . Accordingly we studied the action of a hot solution of sodic ethylate, as follows: Five grams of the dibromdinitrobenzol dissolved in boiling benzol were treated with a hot solution of sodic ethylate in small successive portions. A precipitate was formed immediately as in the reaction with cold sodic ethylate and the liquid turned dark-yellow. After all the sodic ethylate had been added the mixture was boiled for half an hour under a reverse condenser. At the end of this time the precipitate which had formed was filtered out and the dark-yellow filtrate allowed to evaporate spontaneously. The residue was washed with water and the highly-colored wash-waters tested with argentic nitrate and nitric acid, which showed the presence of a bromide in quantity, and with potassic iodide, starch paste and acetic acid, which showed a large amount of nitrite. The remainder of the aqueous solution was acidified with sulphuric acid and extracted with ether, but the amount of oily organic matter thus obtained was too small for investigation. The residue left after washing the product with water was a semi-liquid mass of a terracotta red color, and with a peculiar odor. It was extracted with hot ligroin until only a small tarry residue was left undissolved. On evaporating the dark-yellow ligroin extract a red oil was obtained, which solidified after standing for a short time. After some trouble a satisfactory method of purification was found, consisting in recrystallization from a dilute alcohol, made up of one part of water and three of alcohol, which brought the substance to the constant melting-point 103° to 104° , when it was dried *in vacuo* and analyzed with the following result:

0.3130 gram substance gave 13 cc. N at 22° and 762 mm. pressure.

	Calculated for $C_6H_2BrNO_2(OC_2H_5)_2$	Found.
N	4.82	4.72

The substance contains bromine. These results show that

it must be formed from the dibromdinitrobenzol by the replacement of one nitro group and 1 atom of bromine by two ethoxy groups. The reaction probably takes place in two stages, the first consisting in the replacement of the nitro group to form the phenetol melting at 126° , which is then converted by the replacement of one of the bromine atoms into the body melting at 103° to 104° . This substance must be either the bromnitroresorcin ether, $(OC_2H_5)_{1,3}Br_4NO_6$, or the bromnitropyrocatechin ether, $(OC_2H_5)_{1,2}Br_4NO_5$. In an attempt to determine its constitution we saponified the very small amount of it at our disposal with dilute sulphuric acid, and obtained from the alcoholic solution of the phenol, with an aqueous solution of plumbic acetate, a yellow, flocculent precipitate. This would indicate that the substance is a pyrocatechin ether, but considering the chances of error in this experiment, we cannot place much confidence in it when tried on such a small scale, and as the formation of a resorcin ether is much more probable than that of a pyrocatechin ether, judging from all previous experience in such replacements, we have decided to call our substance provisionally the resorcin ether.

Properties of the 4-Brom-6-nitroresorcin Diethyl Ether, $C_6H_3BrNO_5(OC_2H_5)_2$.—Crystallized from dilute alcohol it forms sheaves of pointed needles of a yellowish-white color. It melts at 103° to 104° , and is freely soluble in benzol, chloroform, or acetone; soluble in ether, glacial acetic acid, or carbonic disulphide; sparingly soluble in ethyl or amyl alcohol, or ligroin, when cold, soluble in each of these liquids when hot. Dilute alcohol containing one part of water to three of alcohol is the best solvent for it. Strong nitric acid has no apparent effect on the substance when cold, but dissolves it slowly when hot; on dilution, what seems to be the original substance, is precipitated. Fuming nitric acid dissolves it at once, even in the cold. Strong sulphuric acid dissolves it with a red color, even in the cold. Sodid hydrate apparently does not react with it, even when hot. Aniline dissolves it in the cold, but gives no change of color even on heating.

The action of an aqueous solution of sodid hydrate with the

1,4 dibrom-2,5-dinitrobenzol was tried on a small scale in order to determine which radicals were replaced, and sodic bromide and sodic nitrite were found among the products of the reaction, from which it appears that the hot solution of sodic hydrate acts like the hot solution of sodic ethylate, so far as the places of attack are concerned. We did not have material enough to study the organic products of the reaction which were contained in the deep blackish-red solution formed by boiling the two reagents together for three hours. At the end of that time all the organic substances had dissolved with the exception of a very little brown tar.

Sodic malonic ester also acted upon the 1,4-dibrom-2,5-dinitrobenzol, forming a red sodium salt, but we did not succeed with the small amount of material we could spare for this work in isolating the substituted malonic ester, as the product obstinately refused to solidify. For this experiment the sodic malonic ester should be made from sodium and the ester, since, if it is made with sodic ethylate in the usual way, the product is in great part the dibromnitrophenetol melting at 126°, already described.

α-Isomer.

Action of Sodic Ethylate on 1,4-Dibrom-2,3-dinitrobenzol (α-Dibromdinitrobenzol).

Five grams of this dibromdinitrobenzol were dissolved in the smallest possible amount of benzol, and mixed with the sodic ethylate prepared by treating 0.7 gram of sodium with 40 cc. of absolute alcohol, that is 2 molecules of ethylate for each molecule of the dibromdinitrobenzol. The mixture gradually took on a dark-red color, and was allowed to stand over night in a corked flask, as it was found that the reaction ran slowly. The liquid mixed with a precipitate which had been formed, was then poured into a dish, and the solvents allowed to evaporate spontaneously. The product, a red, oily mass, was washed three or four times with small quantities of water. The wash-water gave a strong test for a nitrite, a slight test for a bromide. As the residue

did not solidify on standing even at low temperatures, it was extracted several times with boiling ligroin, which left behind a hard, tarry mass of a brick-red color. The solution in ligroin had a lemon-yellow color, and on evaporation left a red oil, which was distilled with steam, when a yellow oil came over, solidifying in the condenser or the receiving-flask. This was filtered out and the filtrate examined for an additional quantity of the product, but it seemed to contain so little that it was not worth while to work it up. The crystalline solid which had come over with the steam was freed from oil by pressing it between pieces of filter-paper, the papers being extracted with ether, and the extract worked up again for the solid substance. As the substance was very soluble in all the common solvents, and its melting-point lay as low as 45° , some trouble was experienced in finding a solvent, which would give crystals. At last, however, we succeeded in purifying it, as follows: A quantity of ligroin (boiling below 70°) was saturated with the substance at a temperature of 50° , and then cooled by immersion in ice, when the body crystallized out in yellow prisms which were washed with ice-cold ligroin and dried between pieces of filter-paper. In this way the substance was obtained with the constant melting-point, 45° , when it was dried *in vacuo* and analyzed with the following results:

I. 0.2153 gram substance gave, by the Carius method, 0.2500 gram argentic bromide.

II. 0.3066 gram substance gave 12.2 cc. N at 18° and 760 mm. pressure. If the determination of nitrogen in this substance is run with only the usual precautions, results more than 2 per cent too high may be obtained. Similar observations have been made frequently in this laboratory in determinations of nitrogen with substances which contain the ethoxy group, and burn with almost explosive violence. The difficulty was overcome, as in previous cases, by distributing the substance through an unusually long layer of fine cupric oxide, and applying the heat with great caution. In this case the greater part of the nitrogen was given off by the heat of conduction before a single burner had been lighted below the layer of oxide and the substance.

	Calculated for $C_6H_2Br_2.NO_2.OC_2H_5$.	I.	Found.	II.
Br	49.23	49.41		
N	4.31			4.59

The substance is, therefore, the 2,5-dibrom-6-nitrophenetol, formed by the replacement of one nitro group by an ethoxy group. Oddly enough, another dibromnitrophenetol has nearly the same melting-point, 46° , but they are not identical, since this was made by Stadel¹ from the 4,6-dibrom-2-nitrophenol. The yield was far from good, 0.9 gram of the uncrystallized substance being obtained from 5 grams of the dibromdinitrobenzol, which amounts to 18 per cent of the theoretical.

Properties of the 2,5-Dibrom-6-nitrophenetol, $C_6H_2Br_2.NO_2.OC_2H_5$.—Crystallized from cold ligroin it forms long, thick, transparent prisms of a lemon-yellow color. It melts at 45° . It is very soluble in all the organic solvents, even when cold; slightly soluble in water. It is left as an oil by the evaporation of its solutions at ordinary temperatures. The best solvent for it is ligroin, from which it crystallizes well, when the solution saturated at 50° is cooled with ice. Strong nitric acid turns the substance red in the cold; on warming, it melts to a red oil, but does not dissolve. Fuming nitric acid dissolves it at once even in the cold, and on dilution white crystals of what seems to be a new substance are precipitated. Strong sulphuric acid has no apparent action, when cold, and on heating the substance melts but does not dissolve. Sodic hydrate does not affect it either cold or hot. Aniline dissolves it, but, even after heating the mixture, what seems to be the original body is recovered when the aniline has been removed by means of dilute hydrochloric acid.

As has been stated already, the 2,5-dibrom-6-nitrophenetol is not formed in very large quantity (less than 18 per cent) by the action of sodic ethylate on the dibromdinitrobenzol. The other products of the reaction must be contained in the aqueous wash-waters and in the tarry residue left on extraction with ligroin, and we devoted a great deal of time to attempts to isolate some definite compound from these products,

¹ Ann. Chem. (Liebig), 217, 57.

but all our experiments led to oily substances which did not solidify on standing, and were obtained in too small quantity for distillation, even although in one case the product came from 20 grams of the dibromdinitrobenzol. As, therefore, we could not be certain of the purity of these substances, we did not waste time in analyzing them.

In order to see whether sodic bromide was formed in addition to sodic nitrite, when 1,4-dibrom-2,3-dinitrobenzol was treated with a boiling instead of a cold solution of sodic ethylate, the following quantitative experiment was tried: Two and a half grams of the dibromdinitrobenzol were boiled for three hours in a flask with a return condenser with a solution of sodic ethylate made from 0.35 gram of sodium. The solvent was allowed to evaporate spontaneously, and the residue, after standing some time, was washed with cold water, which took up a large amount of soluble matter. The washings were acidified with nitric acid, and the rather heavy precipitate filtered out, after which the light-yellow filtrate was precipitated with argentic nitrate. In this way 0.4310 gram of argentic bromide was obtained. This corresponds to 14.95 per cent of all the bromine in the substance, and represents the amount converted into sodic bromide in this process.

A similar quantitative study was made of the action of alcoholic ammonia on the 1,4-dibrom-2,3-dinitrobenzol (α). Two grams of the substance were heated for three hours to 100° in a sealed tube with 20 cc. of alcoholic ammonia, after which the alcohol and excess of ammonia were driven off with a blast of air, and the residue washed with water. Addition of nitric acid to the wash-water produced a precipitate, which was filtered out, and the filtrate was then precipitated with argentic nitrate. In this way 0.3018 gram of argentic bromide was obtained. This corresponds to 13.08 per cent of all the bromine in the substance and represents the amount converted into sodic bromide in this process.

Action of Sodic Hydrate in Solution on the 1,4-Dibrom-2,3-dinitrobenzol (α).

Three grams of the substance were mixed with a solu-

tion of pure sodic hydrate made from 1.2 grams of sodium and 150 cc. of water, and the mixture boiled for seven to eight hours in a flask provided with a return condenser. At the end of this time practically all the solid had disappeared, forming a deep-yellow solution which, after it had cooled, was acidified with sulphuric acid and allowed to stand over night. In order to purify the tarry precipitate with some embedded crystals thus obtained it was distilled with steam, which in the course of a day drove over a considerable amount of a golden-yellow solid. This was filtered out and the filtrate yielded by long extraction with ether nearly an equal amount of the product. The steam distillate melted at 77° , and this melting-point proved to be constant on recrystallization from ligroin. The residue left by the steam in the flask was tarry, and gave only unmanageable substances when treated with solvents. The phenol melting at 77° was dried *in vacuo* and analyzed with the following result:

0.1566 gram of the substance gave, by the Carius method, 0.1996 gram argentic bromide.

	Calculated for $C_6H_2Br_2.NO_2.OH.$	Found.
Br	53.87	54.25

This analysis shows that the body is the 2,5-dibrom-6-nitrophenol formed by the replacement of one nitro by a hydroxyl group, and this inference is confirmed by the analysis of the barium salt given later. The yield was fairly good, as 3 grams gave 1.2 grams of product in one case, 1.35 grams in another, which amounts to about 44 per cent and 49 per cent of the theoretical yield. This replacement of the nitro group is, therefore, probably the principal reaction, and this idea is confirmed by the observation that, whereas much sodic nitrite was formed as a secondary product, the amount of sodic bromide was insignificant. It was also observed that the action of the α -isomer with hot sodic hydrate was much more sluggish than that of the γ -isomer with the same reagent.

Properties of the 2,5-Dibrom-6-nitrophenol, $C_6H_2Br_2.NO_2.OH.$

—It appears in bright golden-yellow needles, which melt at 77° . It is easily soluble in alcohol, methyl alcohol, ether, chloro-

form, acetone, acetic ester, or carbonic disulphide; soluble in benzol; slightly soluble in ligroin, or water. Strong sulphuric acid turned it red, but did not dissolve it even when hot; strong nitric acid had no apparent action in the cold, and when hot it dissolved the substance, but no precipitate was formed on addition of water; hot fuming nitric acid, on the other hand, formed a red solution with it, from which water threw down a precipitate. Aniline turned it red, and, if warmed, gave a red solution from which a red solid was obtained by removing the excess of aniline with hydrochloric acid.

Barium Salt of the 2,5-Dibrom-6-nitrophenol, $(C_6H_2Br_2NO_3)_2Ba_3H_2O$.—This salt was made by boiling the phenol with water in which pure baric carbonate was suspended. The carbonate was decomposed with effervescence and a deep reddish-yellow solution was formed, which was filtered off, and the insoluble part extracted with hot water until the extracts were colorless. The solution was then evaporated until masses of small, yellow crystals separated, which were collected, air-dried, and afterward dried *in vacuo* over sulphuric acid, and then at 100° . Most of the water passed off *in vacuo*, only 1 milligram being lost at 100° from the salt dried *in vacuo*.

0.2100 gram air-dried salt lost 0.0144 gram when heated to 100° .

	Calculated for $(C_6H_2Br_2NO_3)_2Ba_3H_2O$.	Found.
H ₂ O	6.89	6.86
0.1956 gram salt dried at 100° gave 0.0624 gram BaSO ₄ . ¹		

	Calculated for $(C_6H_2Br_2NO_3)_2Ba$.	Found.
Ba	18.84	18.78

The salt, with water of crystallization, forms very small yellow crystals, which turn brown when they lose the water. It is slightly soluble in water.

The action of aniline with the 1,4-dibrom-2,3-dinitrobenzol was tried several times by us, and some of these experiments were on a large scale, when the difficulty of preparing material is considered, but in no case did we succeed in obtaining products which could be brought into a state fit for analysis; we can therefore give no account of this reaction.

¹ It is best to treat the salt with dilute sulphuric acid before treating it with the strong acid.

IV.—ON THE AZOXYBENZALDEHYDES.

By F. J. ALWAY.

In a previous article¹ I have shown that an unidentified compound obtained by Gattermann² is identical with that described by Kirpal as "*p*-azoxybenzaldehyde" and that, when pure, it melts at 190° (uncorr.), as found by Gattermann, the temperature 194°, given by Kirpal,³ being the corrected melting-point, although it was not so stated. In the same article it was mentioned that Lucius and Brüning⁴ had patented a process for the preparation of *p*-azoxybenzaldehyde, *p*-azoxybenzylideneaniline, and homologues of the latter from the corresponding *p*-nitrobenzylaniline, *p*-nitrobenzyltoluidine, etc. The melting-point of the aldehyde and that of its anilide, as given by Lucius and Brüning, were 10° and 18°, respectively, lower than those of the compound of Gattermann and its anilide. It was pointed out further that as the compound of Gattermann was stable toward concentrated sulphuric acid at temperatures as high as 100°, the azoxy nature of the compound was not established. Since sending in the above-mentioned paper it has been found that the compounds obtained by the method of Lucius and Brüning are identical with those already described, and that they may very readily be obtained by the interaction of *p*-nitrobenzyl chloride, aniline or its homologues, alcohol, and caustic alkali under certain conditions. The yield by this method, which is published elsewhere, is 75 to 80 per cent of the theoretical amount, while by the other methods it varies from 10 to 40 per cent of the theoretical. As *p*-nitrobenzyl chloride may be easily prepared by the nitration of benzyl chloride and is much cheaper, if purchased, than *p*-nitrobenzaldehyde, the last-described method is preferable for the preparation of *p*-azoxybenzaldehyde and its derivatives.

The compound melting at 190°, when exposed to the prolonged action of concentrated sulphuric acid at a high tem-

¹ This JOURNAL, 28, 34.

² Ber. d. chem. Ges., 29, 3037 (1896).

³ *Ibid.*, 30, 1597.

⁴ D. R. P., 111, 384.

perature, changes into a compound which has all the ordinary characteristics of the oxyazo compounds. This fact leaves no doubt as to the nature of the compound melting at 190° . It is *p*-azoxybenzaldehyde.

m-Azoxybenzaldehyde is obtained by the action of ferric chloride or of dilute mineral acids upon the electrolytic reduction-products of *m*-nitrobenzaldehyde. It also results when the substance obtained by the action of zinc dust or of amalgamated aluminium upon *m*-nitrobenzaldehyde is exposed to the action of the air, especially in alkaline solution.

EXPERIMENTAL PART.

Conversion of p-Azoxybenzaldehyde into an Oxyazo Compound.

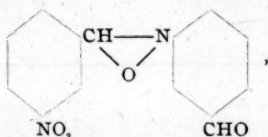
The orange-colored solution of *p*-azoxybenzaldehyde in concentrated sulphuric acid, when heated to 100° and poured into ice-water, yields the unchanged azoxybenzaldehyde. If the temperature be kept at 110° to 120° for some time the solution assumes an intense red color. At the end of an hour and a half the reaction-mixture is poured into cold water. The brown solid which separates out is washed with water and dried on a porous plate. It dissolves in aqueous solutions of ammonia, sodium hydroxide, potassium hydroxide, and alkaline carbonates, forming dark-red solutions. If these solutions come in contact with the skin the latter is dyed yellow. Carbon dioxide, when passed through solutions of the salts, precipitates the brown substance. This dissolves sparingly in alcohol. The alcoholic solution yields, with copper acetate, a brown precipitate which is decomposed by mineral acids. The ammoniacal solution, when treated with an aqueous solution of lead acetate, forms a red, amorphous precipitate. The further investigation of this compound is necessarily interrupted for some months. The reactions indicated are characteristic of the true oxyazo compounds.

Electrolytic Reduction of m-Nitrobenzaldehyde.

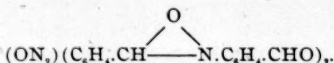
The reduction is effected in the same manner as that of *p*-nitrobenzaldehyde.¹ It is reduced with much more diffi-

¹ This JOURNAL, 28, 34.

culty than the latter. The *N-m*-formylphenyl ether of *m*-nitrobenzaldoxime,



is first formed. By continued reduction this is changed into an insoluble substance, probably an azoxy compound of the formula

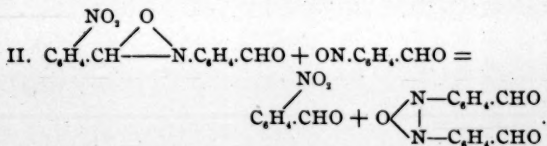
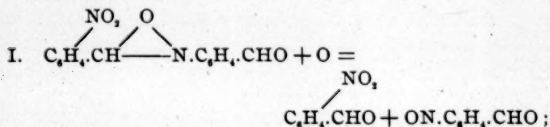


After the current (6 volts, 2 to 3 amperes) has acted six hours a large amount of unchanged *m*-nitrobenzaldehyde remains, together with the primary reduction-product. The filtrate gives no precipitate with sodium carbonate. At the end of fourteen hours the *m*-nitrobenzaldehyde has entirely disappeared and but little of the primary reduction-product remains. In the place of the latter there is a considerable amount of a light-brown, amorphous solid, insoluble in alcohol, acetic acid, and pyridine. This corresponds to the insoluble substance obtained from *p*-nitrobenzaldehyde. In the second case the filtrate yielded a red precipitate with sodium carbonate. The same method that was used with *p*-nitrobenzaldehyde served for the separation of the above crude precipitate into its different components. The *m*-nitrobenzaldehyde dissolved in hot water and was extracted from its aqueous solution by ether. The primary reduction-product, the *N-m*-formylphenyl ether of *m*-nitrobenzaldoxime, dissolves in boiling pyridine, from which the greater part of it separates, on cooling, as a gray, granular solid without distinct crystalline form. The third substance remains undissolved.

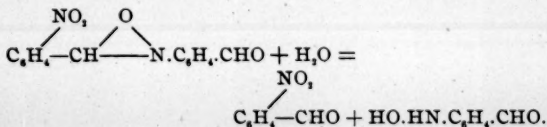
Gattermann¹ has shown that *m*-nitrosobenzaldehyde, *m*-nitrobenzaldehyde and a small amount of an unidentified sub-

¹ Loc. cit.

stance are formed by the action of ferric chloride upon the *N-m*-formylphenyl ether of *m*-nitrobenzaloxime. The third substance I have identified as *m*-azoxybenzaldehyde. Bamberger has shown that nitroso compounds react with hydroxylamine compounds to form azoxy compounds. The probable explanation of the formation of azoxyaldehyde in this case is as follows: The ether is oxidized to nitrobenzaldehyde and nitrosobenzaldehyde. The latter reacts with some of the unchanged ether to form azoxybenzaldehyde. The reactions are expressed by the equations:



When heated with dilute mineral acids the same ether yields *m*-nitrobenzaldehyde and a small amount of *m*-azoxybenzaldehyde. The first reaction is expressed by the equation



The hydroxylaminobenzaldehyde, so formed, is immediately decomposed by the action of the acid, with or without the participation of the oxygen of the air.

The insoluble secondary reduction-product, when oxidized by ferric chloride, gives *m*-nitrosobenzaldehyde and *m*-azoxybenzaldehyde. The latter alone results when the substance is

boiled with dilute mineral acids. In neither case is *m*-nitrobenzaldehyde to be detected.

m-Azoxybenzaldehyde.—This compound may be prepared by several different methods. It is obtained by treating the primary or the secondary reduction-product of *m*-nitrobenzaldehyde with ferric chloride or with dilute mineral acids. It is obtained most conveniently by the following method: The secondary reduction-product or the crude reduction-product obtained by the prolonged action of the electric current upon *m*-nitrobenzaldehyde, is placed in a round-bottomed flask and treated with a ten per cent solution of ferric chloride. Steam is passed through the mixture until *m*-nitrosobenzaldehyde ceases to pass over. The black, tarry substance remaining in the flask is washed with water to free it from ferric chloride and then repeatedly recrystallized from alcohol with addition of animal charcoal. After the fifth or sixth recrystallization the substance is pure.

When *m*-nitrobenzaldehyde is treated with zinc dust and ammonium chloride solution in the cold a yellowish-brown solution is obtained which, when exposed to the air for a considerable length of time, deposits an amorphous, grayish-yellow solid consisting of more or less impure *m*-azoxybenzaldehyde. If the solution be treated with an aqueous alkali it at once assumes a deep-brown color. The brown color rapidly disappears on shaking the mixture, *m*-azoxybenzaldehyde being formed. This is analogous to the formation of *p*-azoxybenzaldehyde.¹ If the insoluble compound $(C_7H_5NO)_x$, described by Bamberger and Friedmann,² be boiled with alcohol and the filtrate be treated with an aqueous alkali, *m*-azoxybenzaldehyde is obtained.

This aldehyde forms almost colorless needles that melt at 129° . It is insoluble in ligroin and cold water, sparingly soluble in ether, hot water, and cold alcohol, but very readily soluble in benzene, acetic acid, and hot alcohol.

¹ *Loc. cit.*

² *Ber. d. chem. Ges.*, 28, 250 (1895).

	Calculated for $C_{14}H_{10}O_2N_2$	I.	Found.
C	66.1	65.7	65.6
H	3.9	4.4	4.5
N	11.0	11.1	...

m-Azoxybenzaldehyde Phenylhydrazone, $(ON_2)(C_6H_4CH:N.NH-C_6H_5)_2$.—This compound is obtained by heating *m*-azoxybenzaldehyde with an excess of phenylhydrazine in alcoholic solution for one hour. Orange-colored crystals separate out. These are filtered out, washed with cold alcohol, and dried on the water-bath. This compound also results by similar treatment of the crude *m*-azoxybenzaldehyde obtained by the zinc dust method. This hydrazone crystallizes in very small, orange-colored needles, melting at 198° .

	Calculated for $C_{20}H_{12}ON_4$	Found.
N	19.3	19.3

Whether *m*-azoxybenzaldehyde can be obtained from *m*-nitrobenzyl chloride by methods analogous to those used in the preparation of *p*-azoxybenzaldehyde has not yet been determined. Attempts to prepare *o*-azoxybenzaldehyde from *o*-nitrobenzyl chloride were not successful. Bamberger¹ has recently obtained the oxime of the last-mentioned compound by another method.

The further investigation of the azoxybenzaldehydes is still in progress in this laboratory.

UNIVERSITY PLACE, NEBRASKA,
JUNE 20, 1902.

Contributions from the Chemical Laboratory of the Rose Polytechnic Institute.

XXII.—CAMPHORIC ACID.

CAMPHANIC AND CAMPHONONIC ACIDS.

[THIRTEENTH PAPER.]

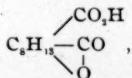
BY WILLIAM A. NOYES AND ROBERT C. WARREN.

The work here described was undertaken for the purpose of securing new evidence as to the structures of camphanic

¹ Ber. d. chem. Ges., 34, 4023 (1901).

and camphononic acids. While the evidence obtained is mainly of a negative character, several new compounds which seem worthy of a brief description have been prepared.

Hydroxycamphoric Acid.—While camphanic acid,



is undoubtedly a lactone, no salt or ester of the corresponding dibasic hydroxy acid has been described. Eleven grams of camphanic acid were heated for twenty-four hours, on the water-bath, with 45 cc. of a 10 per cent solution of sodium hydroxide. By titrating back with nitric acid, using phenolphthaleïn as an indicator, it was found that 96 per cent of the acid had been converted into the sodium salt of hydroxycamphoric acid. The silver salt, $\text{C}_{10}\text{H}_{14}\text{O}_6\text{Ag}_2$, was prepared by precipitation and was crystallized from hot water. The salt gave 50.13 per cent silver. Calculated 50.20 per cent.

The *diethyl ester of hydroxycamphoric acid* was prepared from the silver salt by treating it with ethyl iodide and ether. It is a liquid and has an intensely bitter taste. Its specific gravity is 1.0351 at 20°. The rotatory power is $[\alpha]_D = 40^\circ$ at 20° or 39°.6 at 28°.

The analysis gave :

	Calculated for $\text{C}_{14}\text{H}_{24}\text{O}_6$	I.	Found.	II.
C	61.77	62.02		61.76
H	8.82	8.79		8.85

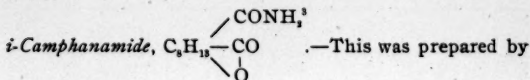
When this work was undertaken it was thought possible by us that the hydroxyl group of this ester was secondary.¹ If this were the case, it should be easy to oxidize it with the formation of the ester of a ketonic acid. It was found, however, that Beckmann's mixture is practically without effect upon it, while nitric acid converts it into the ester of camphanic acid. This furnishes some confirmation of the accepted view, which is that the hydroxyl group is tertiary.

¹ This JOURNAL, 23, 133.

Inactive Camphor.

In a previous paper¹ reference was made to an inactive camphor which is now made synthetically from pinene by the Ampère Chemical Company, of New York. As the method of preparation is of considerable interest and not easily accessible, it seems worth while to give the details as given in the patent.² Five parts of dry turpentine (pinene) and one part of anhydrous oxalic acid are heated to 120° to 130°. There results a mixture of borneol, borneol oxalate, borneol formate, camphor, and waste polymerization-products. The esters of borneol are saponified by heating with milk of lime and the borneol and camphor are distilled with steam. These are separated from oily impurities by freezing and centrifugating, or filtering, and washing. The borneol is oxidized to camphor by potassium pyrochromate and sulphuric acid and the camphor is further purified.

i-Bromcamphoric anhydride.—Camphoric acid is usually treated with 2 molecules of phosphorus pentachloride as the first step in the preparation of bromcamphoric anhydride. As the first effect is to convert the acid to the anhydride it seems more rational to start with the anhydride and use but 1 molecule of the pentachloride. The disagreeable evolution of large quantities of hydrochloric acid mixed with vapors of phosphorus pentachloride and oxychloride is also avoided. One hundred grams of camphoric anhydride, 120 grams of phosphorus pentachloride, and 30 cc. of phosphorus oxychloride were heated on a water-bath in a flask connected with an upright condenser till the mass became thoroughly fluid. The mixture was then cooled, bromine was dropped in, and the remainder of the operation was conducted as described by Aschan,



¹ This Journal, 27, 430.

² No. 698,761, April 29, 1902.

³ Ber. d. chem. Ges., 27, 3505.

treating *i*-bromcamphoric anhydride with a strong, cold solution of ammonia.¹ The amide crystallizes from alcohol in plates or prisms, which melt at 196°. The active amide melts at 208°.¹

The analysis gave :

	Calculated for $C_{10}H_{18}O_2N$.	Found.
C	60.91	60.93
H	7.90	7.84

i-Camphononic acid.—This was prepared by the method used by Lapworth and Lenton² for the preparation of the active acid, with modification in a few particulars. 19.7 grams of camphanic amide were dissolved in 120 cc. of a 10 per cent solution of sodium hydroxide with the aid of heat. By this treatment it is converted into the half amide of hydroxycam-

phoric acid, C_8H_{13} — $\begin{matrix} \diagup CO_2H \\ \text{—} CONH_2 \\ \diagdown OH \end{matrix}$, in which the amide and hydroxyl

groups are combined with the same carbon atom.⁴ The solution was then cooled to 0° and an ice-cold solution of 5.4 cc. of bromine in 120 cc. of sodium hydroxide was added. The temperature rose spontaneously to 25°. Some nitrogen was also evolved. After standing for an hour the solution was heated on the water-bath for an hour. It was then cooled, acidified with hydrochloric acid, some unchanged amide which separated was filtered off, and the filtrate was evaporated nearly to dryness. This treatment transforms the amide group to the amino group and eliminates the latter with the hydrogen of the hydroxyl group, giving the ketonic acid,

C_8H_{13} — $\begin{matrix} \diagup CO_2H \\ \text{=O} \end{matrix}$. The camphononic acid was extracted with

ether, distilled once at a pressure of 15 to 20 mm., and purified by crystallization from a mixture of ligroin and benzene. The yield is poor. The pure, inactive acid melts at 232°.

¹ Auwers and Schnell : Ber. d. chem. Ges., 26, 1526.

² Auwers and Schnell : Loc. cit.

³ J. Chem. Soc. (London), 79, 1283.

⁴ Lapworth and Lenton : Ibid., 79, 1292.

Lapworth gives the melting-point of the active acid as 228°.

The analysis gave :

	Calculated for $C_8H_{14}O_3$.	I.	Found.	II.
C	63.53	63.61		63.34
H	8.24			8.40

i-Camphononic amide, $C_8H_{13} \begin{array}{c} \diagup \text{CONH}_2 \\ \diagdown \text{O} \end{array}$. — Five grams of

i-camphononic acid were heated on a water-bath with 3.2 grams of phosphorus trichloride till solution was complete. The product was cooled and added gradually to 15 cc. of strong ammonia, cooled to -15° . The precipitate was filtered off, dried, and crystallized from benzene. It melts at 215° .

The analysis gave :

	Calculated for $C_8H_{13}O_2N$.	I.	Found.	II.
C	63.90	63.30		63.27
H	8.88	8.89		8.86

It was hoped that by distilling a mixture of *i*-camphononic acid and lime the ketone, $C_8H_{14}O$, recently prepared by one of us with Dr. Patterson,¹ could be obtained. Several grams of the camphononic acid were mixed with twice the weight of lime and the mixture distilled. The distillate possessed the characteristic peppermint-like odor of the ketone, but we did not succeed in securing from it a crystalline oxime.

The following compounds have been prepared by methods similar to those used in the preparation of the corresponding active compounds.

i-Camphoric imide, $C_8H_{14} \begin{array}{c} \diagup \text{CO} \\ \diagdown \text{CO} \end{array} \text{NH}$, crystallizes from water

in needles which melt at 249° . The active imide melts at 245° .

The analysis gave :

¹ This JOURNAL, 27, 424.

	Calculated for $C_{10}H_{16}O_2N_2$.	Found.
C	66.30	66.33
H	8.28	8.53
N	7.73	8.25

i- α -Camphoramidic acid, C_8H_{14} $\begin{matrix} \diagup \text{CONH}_2 \\ \diagdown \text{CO}_2\text{H} \end{matrix}$, was formed chiefly

when 36 grams of *i*-camphoric anhydride suspended in 75 cc. of alcohol were treated with ammonia gas. On cooling, the ammonium salt separated, was filtered off, washed with some alcohol, dissolved in water, and decomposed with hydrochloric acid, added carefully to avoid the formation of a gummy precipitate. The acid was purified by dissolving in concentrated hydrochloric acid and precipitating with water. It then melted at 198°. The active acid melts at 175°. The amide group in this acid is that of the secondary carboxyl. 0.1331 gram of the acid took 7.20 cc. of N/10 potassium hydroxide. Theory requires 7.12 cc.

i-Aminolauronic acid, C_8H_{14} $\begin{matrix} \diagup \text{CO}_2\text{H} \\ \diagdown \text{NH}_2 \end{matrix}$.—The chloride of this

acid was prepared exactly as the chloride of the active acid.¹ It melts with decomposition at 266°. The melting-point of the chloride of the active acid is 304°.

The anhydride of *i*-aminolauronic acid, C_8H_{14} $\begin{matrix} \diagup \text{CO} \\ | \\ \diagdown \text{NH} \end{matrix}$, was prepared by distilling a mixture of the chloride of the acid with twice its weight of lime. It was crystallized from ligroin, in which it is easily soluble. It melts at 209°. The active anhydride melts at 203°.

The analysis gave :

	Calculated for $C_8H_{14}NO$.	Found.
C	70.59	70.82
H	9.81	10.11

i-Nitrosoaminolauronic anhydride, C_8H_{14} $\begin{matrix} \diagup \text{CO} \\ | \\ \diagdown \text{N}-\text{NO} \end{matrix}$.—One and

¹ This JOURNAL, 16, 506.

four-tenths grams of the aminolauroic anhydride were dissolved in 140 cc. of water and 8 cc. of concentrated hydrochloric acid, and the calculated amount of sodium nitrite was added. The mixture was allowed to stand for an hour with occasional stirring, the precipitate was filtered off and crystallized twice from water and once from alcohol. It crystallizes in lemon-yellow prisms which melt at 138°.

The analysis gave :

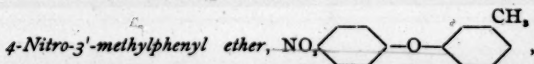
	Calculated for $C_9H_{11}N_2O_2$	Found.
C	59.34	59.54
H	7.69	7.75

TERRE HAUTE, IND., Oct. 18, 1902.

DERIVATIVES OF PHENYL ETHER. IV.

BY ALFRED N. COOK AND GUY G. FRARY.

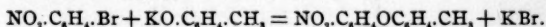
In papers previously published accounts were given of the preparation of several derivatives of phenyl ether by the action of orthobromnitrobenzene upon the ortho, meta, and para-cresolates of potassium, and reducing, oxidizing, and sulphonating the resulting compounds. During the past few months the work has been continued and extended to the reaction between parabromnitrobenzene and the potassium cresolates, with results as given in this paper and two others that are nearly ready for publication.



was prepared by the action of potassium metacresolate (prepared as given in a previous paper¹) on parabromnitrobenzene. Molecular equivalents of the two reacting substances were heated in an oil-bath to 130° when an action began, as was indicated by the ebullition of the fused mass, which continued as long as the temperature was kept at 130°, or above. No rise of temperature was observed due to the reaction itself, as in other cases, but when the containing flask was removed from the bath the reaction ceased. The temperature was

¹ J. Am. Chem. Soc., 23, 810.

kept at 130° to 140° for over two hours, when the reaction seemed to be complete, no action occurring when the temperature was considerably raised. There resulted a copious precipitate of potassium bromide underlying a dark-colored liquid which solidified on cooling. The reaction occurs according to the following equation :



It was not found necessary to extract the substance with ether, wash with caustic soda, and distil with steam, as was done in the earliest work, but it was purified by fractional distillation, directly, under diminished pressure, and then crystallized from hot alcohol, in which it is rather sparingly soluble. The yield was 34 per cent of the theoretical.

4-Nitro-3'-methylphenyl ether is a light-yellow, crystalline substance, which melts at 60° to 61° . It is tasteless but leaves the impression of sulphur when taken into the mouth. Under a pressure of 30 mm. it boils at 230° to 233° . An attempt to oxidize the side-chain by means of chromic acid in acetic acid solution was unsuccessful. The substance was entirely destroyed. Two analyses for nitrogen resulted as follows :

I. 0.3517 gram substance yielded 21.5 cc. N at 23° and 721 mm.

II. 0.3562 gram substance yielded 20 cc. N at 13° and 730 mm.

N	Calculated for	Found.	
	$\text{C}_{13}\text{H}_{11}\text{O}_2\text{N}$.	I.	II.
	6.11	6.54	6.40

Sulphonic acid of 4-nitro-3'-methylphenyl ether, $\text{CH}_3\cdot\text{NO}_2\cdot\text{C}_6\text{H}_4\cdot\text{OSO}_3\text{H}$, was prepared by dissolving some of the corresponding nitromethylphenyl ether in concentrated sulphuric acid by the aid of the heat of the boiling water-bath, diluting with several volumes of water, adding more than sufficient lead nitrate to precipitate the excess of sulphuric acid, precipitating the excess of lead with hydrogen sulphide, and evaporating the solution. When the solution was concentrated to a small bulk the acid separated out as a reddish-yellow, syrupy liquid. When this was dissolved in hot water

and allowed to stand it crystallized out in warty aggregations of fine, white needles. After repeated crystallization it melted at 135° . Some of the salts of the acid were prepared. The composition of the substance was determined by the analysis of the copper salt, given below.

The *barium salt* was prepared by using barium chloride to precipitate the excess of sulphuric acid, instead of the lead nitrate, and sufficient was added also to change all of the sulphonic acid to the barium salt. On concentrating the solution the salt separated out as a fine, white powder. 276.5 parts of the salt are soluble in 1000 parts of water at 80° , and 38 parts are soluble in 1000 parts of water at 24° .

The *strontium salt* was prepared by using strontium nitrate in place of barium chloride, as given above. It was also obtained as a fine, white powder, but it is more soluble than the barium salt.

The *copper salt*, $(\text{CH}_3\text{NO}_2\text{C}_6\text{H}_4\text{O}_2\text{SO}_3)_2\text{Cu} + 4\text{H}_2\text{O}$, was prepared by adding a solution of copper sulphate to a solution of the barium salt in water. The solution of the salt is of a pale-green color. It crystallizes from a water solution in light-green, well-defined crystals. When heated in an air-bath to 100° it loses in weight, as well as its green color, but regains both again by being allowed to come in contact with the air for a short time. On being heated to 160° it changes to a slate color and loses in weight corresponding to 4 molecules of water of crystallization. It is still hygroscopic but does not regain its green color. Two analyses for water and copper resulted as follows:

I. 0.1452 gram salt yielded 0.0160 gram CuO and 0.0134 gram H_2O .

II. 0.1215 gram salt yielded 0.0110 gram H_2O .

	Calculated for $(\text{C}_6\text{H}_4\text{O}_2\text{NS})_2\text{Cu} + 4\text{H}_2\text{O}$	Found.	
		I.	II.
Cu	8.48	8.81	
H_2O (at 100°)	9.58	9.22	9.05

Hydrochloride of 4-amido-3'-methylphenyl ether,

$\text{CH}_3\text{C}_6\text{H}_4\text{OC}_6\text{H}_4\text{NH}_2\text{HCl}$, was prepared by reducing 4-nitro-3'-methylphenyl ether in alcoholic solution with zinc and hy-

drochloric acid while warming on the water-bath. The tin was precipitated with hydrogen sulphide and the filtrate evaporated to crystallization. The substance was somewhat difficult to prepare owing to decomposition taking place while evaporating the solution. It crystallizes out in fine, colorless needles, which shrivel at 146° . An analysis for chlorine resulted as follows:

0.0757 gram hydrochloride yielded 0.0466 gram AgCl.

	Calculated for $C_{13}H_{14}NOCl$.	Found.
Cl	15.07	15.19

The *free amide*, prepared by precipitating from a water solution of the hydrochloride with ammonium hydroxide, is stable when dry but partially decomposes while being desiccated. The *hydronitrate*, prepared by dissolving the free amide in nitric acid, crystallizes readily in feathery crystals on cooling the solution. The *hydrosulphate*, prepared in the same manner, separated out in fine, white crystals. Both the hydronitrate and the hydrosulphate appeared more stable in our hands than the hydrochloride.

Nitro-4-nitro-3'-methylphenyl Ether, $(NO_2)_2 \cdot CH_3 \cdot C_{12}H_7O$.—4-Nitro-3'-methylphenyl ether was treated with concentrated nitric acid in an endeavor to determine its action. On simply mixing the two substances the temperature rose 2° or 3° , and the nitric acid turned to a reddish hue, indicating a slight action at ordinary room temperature. On heating to 90° , however, a violent action began and dense brown fumes of oxides of nitrogen were evolved. The action continued a very few minutes and then ceased. The nitromethylphenyl ether had passed into solution and there remained a dark-red liquid. Continued application of heat apparently did not cause any further action. On pouring this solution into cold water a yellow oil was precipitated which, on repeated washing with water and kneading with a glass rod, became viscous and finally solidified. It was purified by crystallization from alcohol. Nitro-4-nitro-3'-methylphenyl ether is a yellow, crystalline solid which melts at 103° to 104° . The results of the analysis for nitrogen, as well as its mode of formation, would point to its being a dinitromethylphenyl ether.

I. 0.1345 gram substance yielded 13.5 cc. N at 29° and 718 mm.

	Calculated for $C_{13}H_{10}O_5N_2$.	Found.
N	10.22	10.33

MORNINGSIDE COLLEGE,
SIOUX CITY, IA.,
Aug. 22, 1901.

NOTES.

A Rapid Method of Preparation of Crystals of Ammonium Pentasulphide.

By adding to a solution of concentrated ammonium sulphide, which is saturated with sulphur, an equal volume of 95 per cent alcohol I have succeeded in obtaining orange-red, prismatic crystals which form in small rosettes, giving a very beautiful, crystalline formation. The yield is about 60 grams from 200 cc. of ammonium sulphide. The analysis of the crystals was made by the method used by Fritzsche¹ and Sabatier² with the following results:

I. 0.5595 gram crystals dissolved in HCl gave 0.3052 gram NH_4Cl and 0.3605 gram S.

II. 0.3746 gram crystals gave 0.2040 gram NH_4Cl and 0.2423 gram S.

	Calculated for $(NH_4)_2S_5$.	I.	Found.	II.
NH_4	17.35	17.57		17.32
S	65.31	64.43		64.94

Four Carius determinations of total sulphur gave a mean of 81.50 per cent, the calculated amount being 81.63 per cent. The method is so rapid that it suffices to mix the solutions in the evening and set away in a cool place. The crystals are formed by morning. The method will therefore offer an easy way to demonstrate to students of general chemistry the reality of the chemical change involved in the solution of sulphur in ammonium sulphide. Any very wide divergence from the above proportions of alcohol and ammonium sulphide prevents the formation of the crystals.

H. G. BYERS.

UNIV. OF WASHINGTON,
July 18, 1902.

¹ J. prakt. Chem., 32, 313.

² Ann. chim. phys., [5], 22, 73.

The Calibration of Burettes.

Although various excellent methods for calibrating divided tubes, such as burettes, are in use, an addition to the number of one more, possessing far greater simplicity with at least equal precision, may not be considered a work of supererogation.

The method I have to propose depends upon the fact that if a solid body of known volume be immersed in a liquid contained in a burette, the level of the liquid will rise by an amount determined by the volume of the submerged body and the calibre of the tube. As an immersion body I employ a glass rod (see figure), of about 9 mm. diameter, provided, for convenience sake, with a ring at the top and having a volume of exactly 5 cc. The final adjustment of the volume is easily accomplished by grinding the lower end of the rod until it weighs, when suspended in water at 20° C., 4.987 grams less than when weighed in air. This figure comprises in itself corrections for the buoyant effect of air on weights of brass and on the glass, etc. The immersion-body is suspended from the balance by a fine silk thread or by a fine metal wire, 1 cm. of the latter being immersed. An example will make clear the most convenient mode of procedure in getting the right adjustment. Suppose the partly adjusted body is found to weigh in air 12.7300 grams, and in water of 20° C. 7.7155 grams. This result shows that its present loss of weight on immersion is 5.0145 grams, or 0.0276 gram too great, and that its own apparent weight is 0.0700 gram too great.¹ Hence, if it is ground down till it weighs in air 12.6600 grams, it will have the desired volume of 5 true cc. Of course, for any of the varieties of "conventional" cubic centimeters in use, the constant 4.986 will have to be correspondingly modified.



The apparatus is used as follows: The scrupulously clean burette is filled to the point at which it is desired to begin the calibration with distilled water and shaken so as to wet the sides. Forty-five seconds is allowed for the liquid to run down, and its stand is read. Now the immersion-body is let in, suspended on a fine wire or thread of which 1 cm. is under water, and the level again read. Five cc. minus the difference of the two readings is the required correction. The im-

¹ If, A = weight in air and W = weight in water, we have

$$\text{Desired weight} = \frac{4.986}{A-W} A$$

mersion-body may now be removed and dried and the entire operation repeated for the succeeding 5 cc. There are no appreciable sources of error beyond those of reading the burette. If the glass is perfectly clear there need be no fear of error from air bubbles adhering to the immersion-body. The method has been used for about two years in this laboratory by myself and many students with much satisfaction.

CHEMICAL LABORATORY OF THE
UNIV. OF IOWA, IOWA CITY,
JANUARY 22, 1902.

LAUNCELOT W. ANDREWS.

A Convenient Gas Generator.

In a recent letter to the editor, Dr. R. M. Bird, of the Mississippi Agricultural and Mechanical College, suggested a very simple apparatus for obtaining a supply of gas for two or three Bunsen burners by saturating air with gasoline vapor. The accompanying cut shows the main features of the apparatus. The air-blast is obtained by means of a filter-pump arranged as shown in Fig. I. The 2-liter bottle should be

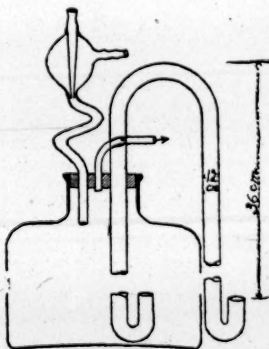


Fig. I.

provided with an outlet tube sufficiently large to prevent flooding the apparatus. If there is not an adequate water supply, a tank may be filled with compressed air by means of a bicycle foot-pump. The saturator, *S*, Fig. II., was made from a broken condenser-jacket, and contained a rolled-up

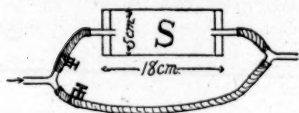


Fig. II.

towel saturated with gasoline. The side tube was added in order to regulate the flow of gasoline to the burner. A single filling with gasoline supplied two burners for a considerable time, giving good-sized, steady flames. A blast-lamp could be used with the apparatus.

R. M. BIRD.

REVIEWS.

TEXT-BOOK OF PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY. By G. BUNGE, Professor of Physiological Chemistry at Bâle. Second English edition, translated from the fourth German edition by FLORENCE A. STARLING and edited by ERNEST H. STARLING, M.D., F.R.S., Professor of Physiology in University College, London, England. Philadelphia: P. Blakiston's Son & Co. 1902. 8vo. xi + 470 pp. Price, \$3.00 net.

Professor Bunge's lectures on physiological chemistry have had a great influence on physiological chemistry both here and abroad. Representing as they do the ideas which have produced, throughout many years, discoveries of fundamental importance in the school of Schmiedeberg, they have served to spread the method of thought of that school and to render more effective the work of men in other laboratories. These lectures have also the merit of being written by a man who was a philosopher and chemist before he was a physiologist, and who, being thus in a position to grasp the general bearings of his subject, has succeeded in making the dry bones of physiological chemistry interesting even to the beginner.

The author's recommendation to students to go back whenever possible to the original papers is endorsed by the editor, and copious references enable the student to do this very readily. The following statement of the editor will meet the hearty approval of all teachers of physiological chemistry: "A careful study of a few of the classic researches in their original form will do more to acquaint a man with the spirit of physiology than the most arduous perusal of text-books. It is essential to the healthy development of the thinking powers that they should have some work to do, and not be nourished solely on a diet of already digested material."

Professor Bunge is an advocate of the vitalistic theory and occasionally his conclusions are not those which commend themselves to the majority of physiologists. The editor has indicated in foot-notes other opinions and has distinguished such additions by brackets.

The translation is well done and the book is provided with an excellent index.

W. R. O.

METHODS OF GAS ANALYSIS. By DR. WALTHER HEMPEL, Professor of Chemistry in the Dresden Technische Hochschule. Translated from the third German edition and considerably enlarged by L. M. DENNIS, Professor of Analytical and Inorganic Chemistry in Cornell University. New York: The Macmillan Co. 1902. Price, \$2.25.

The great value of this book as a guide in all gas analysis is so well recognized that it is only necessary to call attention to the publication of this edition which "has been thoroughly revised by both the author and the translator, and has been changed to such an extent that this translation may indeed be regarded as a fourth edition of the book."

"The additions comprise several new methods of collecting and keeping gas samples; determinations with the Honigmann, Bunte, and Orsat apparatus; the new Hempel methods for exact gas analysis; new methods for the determination of combustible gases; the separation of argon from the atmosphere; improved methods for the determination of carbon monoxide in gas mixtures; the analysis of acetylene gas; the examination of gases produced by living bacteria; the simultaneous determination of fluorine and carbon dioxide; the determination of the heating power of gases; sulphur in organic substances; the gas lantern; the volumetric determination of carbon in iron; the analysis of gases evolved in the electrolysis of chlorides and in the manufacture of bleaching-powder, together with many minor changes and additions throughout the book."

J. R. G.

Corrections.

Page 4, line 7. For "base" read "bore."

Page 13, line 2 from bottom. For "collar" read "ring."